ANNUAL RESEARCH PROGRESS REPORT

FY 1998

Grand Forks Human Nutrition Research Center

ANNUAL RESEARCH PROGRESS REPORT

(FY 1998)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA

GRAND FORKS, NORTH DAKOTA 58202

MINERAL NUTRIENT FUNCTIONS

MANAGEMENT UNIT

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149753 Year: 98 Project Number: 5450-51000-012-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: EFFECTS OF COPPER DEPLETION ON CARDIOVASCULAR FUNCTION AND METABOLISM

Period Covered From: 03/96 To: 03/01

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

Diets in the U.S. frequently are low in copper when they are compared to dietary standards set by the National Research Council of the National Academy of Sciences. Although 1.5 to 3 mg are recommended as a daily safe and adequate range of intake for adults, only 60% of diets in a large analytical survey exceeded the lower limit of this range and approximately one third of the diets were less than 1 mg daily. Daily amounts of copper less than 1 mg have been proved insufficient for men and women in well-controlled dietary experiments. These experiments revealed that people respond to diets low in copper similarly to several species of animals with, among other things, abnormal electrocardiograms, increased cholesterol in blood, impaired metabolism of sugar, and poor control of blood pressure.

Dietary requirements for copper and related mineral elements are defined best by experiments involving human volunteers. Because experiments with humans cost hundreds of thousands of dollars, and certain procedures are either unethical or intolerable, occasional human studies are supplemented with numerous experiments with a variety of experimental animals. These experiments generally compare low intake of copper, for example, with a higher intake. Ideally, the low intake is one consumed by some people who choose their own diets and the higher intake is close to dietary standards. If biochemical or physiological measurements are abnormal at the lower intake, this intake is defined as inadequate and the higher intake is identified as preventing these changes. Animal experiments of this type identify new functions of nutrients and elucidate mechanisms of action. Experiments both with people and with animals are used to establish national dietary standards.

How serious is the problem? Why does it matter?

The major signs of copper deficiency found in depleted men and women and deficient animals resemble the most common characteristics that can predict risk of ischemic heart disease in people. More than 70 anatomical, chemical and physiological similarities between animals deficient in copper and people with ischemic heart disease have been identified. It seems likely that the low copper diet common in the U.S. contributes to this disease which is the leading cause of death in the U.S., 480,000 deaths

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annually. The cost of medical care for this illness is more than $5 billion per year which does not include either effects of sorrow, time lost from work or annual cost of prevention (at least $1000 per person). Proper selection of foods may yield diets that meet the standards mentioned above and prevent both illness and expense.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

There is no doubt that ischemic heart disease is mainly of dietary origin because people who immigrate and change their diets gradually acquire the risk of people born in their new country. Animal protein and fat are the major dietary components associated with heart disease world wide. Animal experiments reveal that adequate dietary copper protects against adverse effects of diets high in fat. The goal of this work is related to National Program 107 Human Nutrition Requirements, Food Composition and Intake equally divided between two components: a. Develop information about the effects of mild deficiency or imbalance of specific nutrients on biochemical, physiologic, and psychological functions to facilitate their detection and prevention and to define requirements for optimal health and performance for adults during the prime and latter years of life and b. Establish safe and optimal levels for dietary components and their roles in risk or prevention of diet-related disorders to provide comprehensive information about the effect of diet on chronic disease risk.

What was your most significant accomplishment this past year?

Although official recommendations about desirable amounts of dietary copper have been made (See Question 1), no Recommended Dietary Allowance (RDA) has been assigned. The first publication listed below explains the importance of assigning an RDA. 1) Nutrients without RDAs often receive little attention in dietary surveys, advice on food selection, nutrition information, dietary planning and nutritional research. 2) Human depletion experiments with copper are more numerous than for magnesium, selenium and zinc, all of which have RDAs; therefore evidence for a copper RDA is sufficient. 3) In addition to the link of low dietary copper to ischemic heart disease (See Question 2), the recently identified link between heart disease and osteoporosis should prompt a greater consideration of copper and health. The finding of fragile bones in mice deficient in copper (fourth publication in list) confirms this link. Adequate dietary copper is required for health of both hearts and bones.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

Before this project began nearly all sources of nutritional information stated that "adults generally consume 2 to 5 mg of copper daily, an ample

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amount as diets of otherwise mediocre quality contain enough copper." A disparity between actual copper intakes and both copper requirements and dietary recommendations has been recognized. This recognition culminated in publication by this author of dietary data pooled from 10 research groups in four countries to reveal dietary facts summarized in Question 1, above. History of the copper RDA (see Question 4), criteria and new paradigms related to human heart and bone disease were co-authored.

High blood pressure from copper deficiency may be explained partially by the finding of impaired defense against damage by oxygen such as cholesterol oxidation and a related impaired ability to relax blood vessels.

Defense against oxidative damage that depends on adequate dietary copper is impaired in copper deficiency, where, however, genetic control of defense based on manganese is improved. This finding may identify a partially compensating mechanism.

Aortic aneurysms are a leading cause of death in men over age 50; low copper diets may contribute to aneurysm development (aneurysms are pathological enlargements of arteries that sometimes burst, an event that can be fatal).

Recent work on the effects of insufficient dietary copper in people resembles work by Goldberger on pellagra early in this century. Pellagra, a deficiency of niacin, was nearly as common then as heart disease is now; early dietary depletion experiments with people had similar success rates.

All of these findings are related to the mechanisms by which the Western diet gradually damages arteries and hearts leading to ischemic heart disease (see Question 2). Improving diets low in copper by selection of foods high in copper and by decreasing intake of foods low in copper is likely to have great benefits to both health and to decreasing the vast annual expenditures on medical care in middle and old age (see Question 2). Regular consumption of diets adequate in copper may lengthen life and assist in attaining a healthier old age. The ARS Food Pyramid is a useful guide.

What do you expect to accomplish during the next year?

A study of obese women who lose weight because of exercise and restricted food intake will be conducted. Beneficial effects of copper supplements on cholesterol metabolism and bone density will be sought. Effects of copper supplements on heart rhythm of women low in copper will be sought using the state-of-the-art computer technique of spectral analysis in the evaluation of electrocardiograms recorded in past studies. An interactive effect of dietary copper and dietary magnesium will be sought by studying the frequency of abnormal heart beats during several hours of electrocardiographic recording.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durability of the technology

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Several medical practitioners, graduate students, reporters for a radio news program and Cable News Network, and a U.S. Senator's office enquired via the telephone and were given information about dietary copper, hazards of low copper intakes, drinking water quality, high copper foods and the hazards of large zinc supplements and of environmental cadmium. This information was used upon receipt. A textbook on trace element metabolism in humans is being edited.

Rodale Press soon will publish a book "Natures Medicines" which will disseminate our findings on copper to a wide audience. Much of this dissemination will include our work that led up to this project: the induction in people of high cholesterol and abnormal electrocardiograms with a diet low in copper along with potentially adverse effects on sugar metabolism and blood pressure. According to experiments with animals, these effects can occur even when copper deficiency is too mild to be detected by the usual biochemical or clinical indices.

The United States Pharmacopeial Convention (USP) provides standards to the Food and Drug Administration in efficacy, quality and safety, etc., of prescription medicines. Dr. Klevay is a member of the USP Advisory Panel of Nutrition and Electrolytes which writes approximately a dozen nutritional monographs each year. These are incorporated into annually revised volumes published by the USP being immediately available to physicians and pharmacists and other regulatory agencies such as state medical boards.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Over the years, work leading up to this project has received favorable mention in well over 100 newspapers and magazines here and abroad. In the past year Health, Dallas Morning News and Agriculture Research published favorable summaries on this project.

"Nutrition and Heart Disease" as presented to subscribers of the Interactive Video Network sponsored by North Dakota State University.

An article on some potential hazards of dietary supplements was published in the Grand Forks Herald. Emphasis was placed on supplements based on botanicals because of known problems of contamination and poor data on desirable dose.

Publications:

01. KLEVAY, L.M. 1998. Lack of a recommended dietary allowance for copper may be hazrdous to your health. J. Am. Coll. Nutr. 17:322-326.

02. KLEVAY, L.M. 1998. Letter to the Editor. J. Trace Elements Med. Biol. 12:1.

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Publications: (Continued)

03. KLEVAY, L.M., CHRISTOPHERSON, D.M. and SHULER, T.R. 1998. Multiple nutritional elements in hair of one man over two decades. FASEB J. 12:A227.

04. WILDMAN, R.E.C. and KLEVAY, L.M. 1998. Decreased bone integrity in mice with meat anemia. FASEB J. 12:A219.

05. KLEVAY, L.M., et.al. 1998. A diet high in whole and refined foods decreases cholesterol oxidation...and selenium. Food, Phytonutrients, and Hlth Forum/ Workshop, Wash DC, Mar 9-11.

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400377 Year: 98 Project Number: 5450-51000-020-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: HOMEOSTASIS AND BIOAVAILABILITY OF TRACE ELEMENTS IN HUMANS AND ANIMALS

Period Covered From: 04/96 To: 04/01

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

A. Manganese is an essential element, and domestic animals deficient in manganese have severe health problems (skeletal and developmental abnormalities). Although there are only a few reports of possible manganese deficiency in humans, there is some evidence that deficiency may predispose humans to seizures, cancer and bone problems. Manganese is also a toxic element when consumed in excess, and it is possible that excess manganese may lead to neurological problems, and perhaps violence. However, very few studies supporting or refuting the above possibilities have been conducted in healthy people consuming normal Western diets. Thus our questions are: Is there concern for either a deficiency or excess of manganese in humans consuming normal Western diets? What factors exacerbate or inhibit the uptake and/or retention of manganese from meals?
Approach: Animals are being used to study the mechanisms of manganese absorption and factors that influence absorption. A series of studies is examining the interaction between manganese and iron and how that interaction affects manganese absorption. Animals are being used to study how manganese is distributed in the body, especially when manganese is consumed in luxurious amounts. Animals are also being used to study how the excretion of manganese is regulated, and how variable excretion is used to prevent a build-up of manganese in the body. Human studies are conducted to confirm findings generated from animal studies. Human studies examine factors that interact to affect manganese absorption, and human studies also examine markers of health problems associated with low or high intakes of manganese.
B. Selenium is found in variable amounts and chemical forms in different foods, and the potential health benefits of selenium depend on the amount and chemical form of the selenium consumed. Areas of the Northern Plains of the U.S. have high concentrations of selenium in the soil that result in high concentrations of selenium in agricultural products such as beef and wheat. The focus of this research is to examine the health benefits, especially the prevention of colon cancer, of different foods that contain high concentrations of selenium. The foods we have concentrated on are wheat, meat and broccoli. Wheat and meat are being studied because they are the single biggest source of selenium in the Western diet. Broccoli is being studied because it contains selenium in a form that may be especially protective against colon cancer.
Approach: Animals are being used to study the effectiveness of different food forms of selenium in preventing Aberrant Crypt Foci (ACF), a marker

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National Program(s): 107 100%

that is highly associated with the development of colon cancer. Animals are also being used to study the mechanism by which selenium inhibits ACF. Human studies will involve the feeding of stable isotopes of selenium incorporated into foods, and then following the retention and excretion of the isotope.

How serious is the problem? Why does it matter?

The studies with manganese are important for two reasons. First, manganese is an essential element and manganese deficiency causes severe problems, such as bone and skeletal abnormalities, in domestic and wild animals. If animals are so susceptible to manganese deficiency, then humans may also be susceptible. However, because of a lack of well designed studies, a Recommended Dietary Allowance (RDA) has not been established for manganese. These studies should give information as to the nutritional importance and/or need of establishing an RDA for this element.

Second, manganese is toxic in large amounts, and some have suggested that even dietary amounts may be harmful, and all traces of the element should be removed from food. Data from our studies are showing that the body very effectively regulates manganese retention, and consequently amounts commonly encountered in foods - amounts less than those needed to create overt toxicity - are not cause for concern.

The selenium research is important because of the reported dramatic cancer-preventive effects of this element. As a consequence of the human cancer studies, it is possible that selenium intakes of up to 200 ug/d may be recommended to the general public. Our research will determine types of food that will provide risk amounts of the most effective form of selenium in its cancer preventative role.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

These studies relate directly to National Program 107, Human Nutrition Requirements. They specifically relate to the components defining marginal or borderline deficiencies of nutrients and determining the bioavailability of nutrients from foods.

What was your most significant accomplishment this past year?

We have demonstrated that selenium is effective in preventing the development of preneoplastic colon cancer lesions (ACF) in rats. We showed that the reduction in ACF was dependent on the amount and chemical form of dietary selenium. Other studies showed that the form of selenium in broccoli may be the most effective, as well as the safest, form of selenium to be used as a supplement.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

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1. Studies examining the health benefits of selenium have shown that supplemental selenium improves the mood of healthy young men. This effect was seen in selenium adequate and selenium deficient men. This finding shows the necessity of selenium for proper brain function, and provides an additional reason to maintain proper selenium nutriture.
2. Aberrant crypt foci (ACF) have been shown to be reduced in selenium-low rats fed selenium, and the magnitude of the effect depends on the amount and chemical form of the selenium supplied. The reduction in ACF most likely ocurred because selenium decreases the number of DNA adducts in the colon. These studies add more evidence to the hypothesis that selenium may decrease the risk of colon cancer.
3. Studies with broccoli raised to contain high concentrations of selenium showed that humans and animals retain and distribute selenium from broccoli differently than selenium salts such as selenite and selenate, and from selenium amino acids such as selenomethionine and selenocysteine. The selenium from broccoli may be less toxic, and more efficacious in prevention of colon cancer. These findings may show that selenium from broccoli may be a superior source for obtaining supplemental selenium.
4. Studies have characterized the cellular aspects of manganese absorption and have shown that manganese is quickly moved by cultured gut cells in a direction analogous to excretion into the gut lumen, whereas movement in the direction of absorption is slow and apparently by diffusion.
5. Studies have shown the importance of iron nutriture on the absorption of manganese. A human study tested, and confirmed, the hypothesis that iron stores are a primary determinant of the amount of manganese absorbed from a meal. Animal experiments have shown that saturated fat decreases manganese absorption and that animals defective in a protein that binds iron on the surface of the gut cell absorb more manganese than normal animals.

What do you expect to accomplish during the next year?

Healthy women will be fed diets containing manganese in the highest or lowest amounts that can be normally encountered in foods. Health effects will be carefully monitored, especially for signs of neurological problems indicative of potential toxicity, as well as for any deleterious effects of low manganese.

More studies will be conducted to determine the comparative effectiveness of beef, wheat and broccoli as sources of supplemental selenium. Animal studies will determine the effect of these foods on the molecular regulation of selenoproteins. Other animal studies will examine the nature of the cancer-preventive effects; emphasis will be placed on the regulation of apoptosis and the suppression of DNA adduct formation.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

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Information from the studies in our laboratory has been passed on to customers of the Grand Forks Human Nutrition Research Center. A primary customer is the scientific community and information has been passed to this customer by the publishing of scientific articles. Wheat and meat are the primary sources of selenium in the diet, thus presentation to the general public of the health benefits of selenium may be beneficial to producers of these commodities. A portion of this work with beef is funded by the North Dakota Beef Commission, and they have received a preliminary report of this information. Interest in the possible enhancement of commodity value has also resulted in an invitation to present information at the N.D. Marketplace of Agricultural Ideas, to be held in Bismarck, ND, in January, 1999. Interest in the health benefits of selenium in beef have led to an invitation to talk at a symposium entitled "Health benefits of phytonutrients, possible bio-accumulation in animal products" at the American Association of Animal Scientists in June of 1999. Interest in the potential health benefits of selenium in wheat resulted in an invitation to present those findings at General Mills, Inc., in Minneapolis, MN in August of 1998.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

J. Finley presented two seminars entitled "Manganese deficiency and toxicity: Is there concern of either for healthy humans consuming normal diets?", and "The efficacy of various forms of selenium in prevention of colon cancer" at the Departments of Animal Science and Human Nutrition at Washington State University in May of 1998.
J. Finley presented a seminar entitled "The anti-cancer benefits of Se - Is wheat an effective supplemental source?" to General Mills Inc., in August of 1998.
J. Finley presented an interactive video seminar to North Dakota state dieticians entitled "Mineral essentiality in healthy adults, are dietary intakes adequate?"

Publications:

01. FINLEY, J.W. 1998. Manganese uptake and release by cultured human hepato-carcinoma (Hep-G2) cells. Biol. Trace Elem. Res. 64:101-118.

02. DAVIS, C.D., SCHAFER, D.M. and FINLEY, J.W. 1998. Effect of biliary ligation on manganese accumulation in rat brain. Biol. Trace Elem. Res. 64:61-74.

03. FINLEY, J.W., CATON, J.S., ZHOU, Z. and DAVISON, K.L. 1997. A surgical model for determination of true absorption and biliary excretion of manganese in conscious swine fed commercial diets. J. Nutr. 127:2334-2341.

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National Program(s): 107 100%

Publications: (Continued)

04. MARENTES, E., VANDERPOOL, R.A. and SHELP, B.J. 1997. Boron-isotope fractionation in plants. Can. J. Plant Sci. 77:627-629.

05. FINLEY, J.W. and PENLAND, J.G. 1998. Adequacy or deprivation of dietary selenium in health men: Clinical and psychological findings. J. Trace Elem. Exp. Med. 11:11-27.

06. FINLEY, J.W. 1998. The absorption and tissue distribution of seleniium from high-selenium broccoli are different from...and selenomethionine as determined in selenium-deficient rats. J. Agricul. Food Chem 46:3702-3707.

07. FINLEY, J., DAVIS, C. and FENG, Y. 1998. Nutritional bioavailability of selenium in broccoli. FASEB J. 12:A823.

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401877 Year: 98 Project Number: 5450-51000-020-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: SELENIUM IN NORTH DAKOTA BEEF

Period Covered From: 06/98 To: 12/98

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

Selenium is found in variable amounts and chemical forms in different foods, and the potential health benefits of selenium depend on the amount and chemical form of the selenium consumed. Areas of the Northern Plains of the U.S. have high concentrations of selenium in the soil that may result in high concentrations of selenium in agricultural products such as beef and wheat. If areas that produce high-selenium agricultural products can be identified, then it may be possible to enhance the marketability and/or profitability of food products from these areas promoting the health effects of the selenium they contain.
Approach: There are a few reports of high concentrations of selenium in grasses in several areas of North Dakota. Additionally, soil maps indicate where soil selenium is potentially high. Based on this initial information, a study was set up that examined the soil and plant selenium content of these areas, and also determined whether beef grown in these areas was high in selenium. Plant and soil samples were collected in the field, and older cows from the same areas were shipped to a slaughterhouse where muscle samples were obtained.

How serious is the problem? Why does it matter?

The selenium research is important because of the reported profound cancer-preventive effects of this element. As a consequence of the human cancer studies, it is possible that selenium intakes of up to 200 ug/d may be recommended to the general public. Our research will determine whether beef from North Dakota may be a potential rich source of supplemental selenium.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

These studies relate directly to National Program 107, Human Nutrition Requirements. They specifically relate to the component concerned with determining the bioavailability of nutrients from foods.

What was your most significant accomplishment this past year?

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National Program(s): 107 100%

This project began in the past year, and currently the fieldwork (i.e. grass, soil and animal sampling) has been completed. Sample analysis is currently underway.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

It is predicted that beef from some areas of North Dakota will have selenium concentrations of five to twenty-fold that of beef grown in moderate or low selenium areas of the country. It is predicted that this information may allow such producers to market part of their animals in a specialty market, thus enhancing their profit. Enhancing the profit of beef producers is important because of the low prices they are currently receiving for their product.

What do you expect to accomplish during the next year?

During the next year, we plan to finish analysis of all samples collected during the survey of the state. We also plan to conduct controlled feeding studies to determine whether the selenium content of beef can be enhanced by feeding feedstuffs such as wheat and hay that are naturally enriched in selenium.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

Information from these studies is being passed on to customers of the Grand Forks Human Nutrition Research Center. The North Dakota Beef Commission and the North Dakota Agricultural Products Utilization Commission has been given a report of our activities and preliminary findings. A seminar entitled "Selenium in wheat and meat in North Dakota: Can it be used to enhance the profitability and/or marketability of these products?" was presented at the North Dakota Marketplace symposium. Interest in the health benefits of selenium in beef have led to an invitation to talk at a symposium entitled "Health benefits of phytonutrients, possible bio-accumulation in animal products" at the American Association of Animal Scientists in June of 1999. Interest in the potential health benefits of selenium in wheat resulted in an invitation to present those findings at General Mills, Inc., in Minneapolis, MN in August of 1998.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

J. Finley presented a seminar entitled "Selenium in wheat and meat in

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North Dakota: Can it be used to enhance the profitability and/or marketability of these products?" at Marketplace 1999 in Bismarck, N.D. in January of 1999.

Publications:

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0400398 Year: 98 Project Number: 5450-51000-021-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: BIOAVAILABILITY OF TRACE ELEMENTS, ESPECIALLY IRON FROM FOOD, & ITS INFLUENCE ON NUTRITURE & FUNCTION

Period Covered From: 04/96 To: 03/01

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

Iron bioavailability, depending on the form of iron, and the presence of enhancers or inhibitors in foods, can vary 5-10 fold from meals with similar iron content, but does not substantially affect iron stores after such diets are fed for several weeks. How should this apparent discrepancy affect dietary advice for the public?
Both short-term iron absorption and longer-term iron status are being measured in humans consuming controlled diets for several weeks to help determine the true importance of dietary iron bioavailability, and the related impact on practical dietary choices such as eating less meat, more beans and whole grains, or more tea. The bioavailability of other mineral nutrients, such as calcium, copper, and zinc, may also be affected by such dietary choices, and information on bioavailability of these other mineral nutrients can often be efficiently derived from the same human studies. The iron research will determine the practical importance of dietary iron bioavailability, and how extensively biological adaptation modifies it.

How serious is the problem? Why does it matter?

Iron nutrition is a double-edged sword. While adequate iron nutriture in infants, children, and childbearing age women is essential for cognitive development and work capacity, excessive body iron in men and postmenopausal women is hypothesized to increase oxidative stress and the risk of chronic diseases such as heart disease and cancer. Research on dietary iron bioavailability provides information for policy decisions about recommended dietary allowances, food enrichment and fortification standards, and dietary guidelines for the public.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition, and Intake (100%)
This research is directly related to one of ten current nutrition problems and related objectives designated in the ARS National Programs.
Specifically, this research concerns the problem, "the bioavailability of nutrients in food".

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What was your most significant accomplishment this past year?

Demonstrated that US men partially adapt to differences in dietary iron bioavailability, despite the high iron bioavailability of their customary diet. Five-fold differences in the nonheme iron absorbed from diets with high and low iron bioavailability were reduced to just over 2-fold after 10 weeks. Interestingly, adaptation occurred without a change in serum ferritin, the best noninvasive indicator of body iron stores in humans. These results also demonstrated that research with short-term diets overestimates differences in iron bioavailability between chronic diets. This information can be helpful in setting recommended dietary allowances for iron.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

The accomplishment mentioned above is probably the most important to date. Additional accomplishments follow.
Determined that women of child-bearing age absorbed substantially less (about 70% less) nonheme iron from a lacto-ovo-vegetarian, compared with an omnivorous diet. Documented that common measures of iron status were unaffected by consuming such a diet for eight weeks, but that fecal ferritin excretion was sensitively affected by dietary iron bioavailability. A paper has been submitted for publication. These findings emphasize the limited response of serum ferritin (a proposed risk factor for cardiovascular diseases and cancer) to dietary iron bioavailability, as well as providing a basis for dietary advice to vegetarians.
A surface response study demonstrated that copper, iron and zinc interact to influence body mineral status, that high dietary iron does not induce overt oxidative stress, and that indices of antioxidant capacity are primarily influenced by dietary copper in rats. Publication is in progress. These results will help evaluate the impact of dietary iron on risk factors for chronic disease.
Accomplishments on research still in progress: An study was designed to extend to women the research on adaptation to iron bioavailability. A 3-year study was designed and initial samples were collected to quantify human iron excretion in relation to gender and body iron stores. Research was designed to determine the effect of body iron stores and of dietary calcium on mucosal uptake and serosal transfer of both heme and nonheme iron. Sample collection was begun to determine the influence of iron supplementation on adaptation in iron absorption by adult men and women consuming a beef meal (see report for CRIS 5450-51000-021-03T). Stable copper isotope analyses were completed in a study to determine if previous observations of reduced serum copper and ceruloplasmin associated with a lactoovovegetarian diet can be explained by reduced copper absorption from copper-abundant vegetarian diets. Sample collection was completed on 300

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400398 Year: 98 Project Number: 5450-51000-021-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

women in an experiment to test the strength of association between iron stores and dietary factors such as meat intake, using more extensive dietary assessment data than has commonly been applied. This research will determine whether dietary variables that affect short-term dietary bioavailability can be related to long-term iron stores. An investigation was begun to determine the role of beta-2 microglobulin in intestinal mucosal uptake, body retention, and organ distribution of iron and manganese, using the beta-2 microglobulin knockout mouse and radioisotopic tracers. Mice with a mutation in this protein exhibit similarities to human hemochromatosis, a disease of excess iron storage.

What do you expect to accomplish during the next year?

Research in the coming year will advance phases of the studies mentioned above. We will determine if women of childbearing age adapt to dietary iron bioavailability, and whether adaptation is limited to the specific dietary enhancers and inhibitors in the chronic diet, or constitutes a more general change in absorptive efficiency. We will also complete data analyses to determine the effect of a vegetarian diet on copper absorption in women. New research will be designed to test the hypothesis that, unlike purified sources of protein, meat consumption does not reduce body calcium retention.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

Because of research on iron and zinc bioavailability and requirements, JR Hunt was invited to participate in an Ad Hoc Expert Panel to prepare the report: "Analysis and Review of Available Data and Expert Opinion on the Potential Value of Vitamin and Mineral Supplements to Meet Nutrient Gaps Among Low-Income Individuals", available from Life Sciences Research Office, Bethesda, MD. This report will be part of a larger USDA report to Congress on the use of food stamps to purchase vitamins and mineral supplements, scheduled for release December 15, 1998.
A preliminary report of the research showing that US men partially adapt to differences in dietary iron bioavailability was submitted to the North Dakota Beef Commission, which partially sponsored the research. Results were also presented at the national Experimental Biology meeting, and the South Dakota State University's 15th Annual Nutrition Seminar. A technical manuscript is currently in preparation.
Research cited in the USDA-ARS Food and Nutrition Research Briefs, "Foods Don't Trigger Calcium Loss", attracted attention from both news and agricultural groups, including representatives from The Medical Post, Toronto, Ontario; "Nutrition & the MD" newsletter, Hagerstown, MD; and the Asian Nutrition Newsletter published quarterly by the Meat & Livestock Association of Australia. Follow-up research is needed to more definitively

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0400398 Year: 98 Project Number: 5450-51000-021-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

address this current controversy in nutrition, and more has been funded through the Post-doctoral Research Associate program for 1999.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

The front page New York Times article, "In Vitamin Mania, Millions Take a Gamble on Health", by Jane E. Brody, October 26, 1997, extensively cited a paper written by JR Hunt: Position of the American Dietetic Association: Vitamin and mineral supplementation. J Am Dietet Assoc 96:73-77, 1996.
JR Hunt organized the meeting "Benefits of Nutrition Research for Native Americans" between the GFHNRC and local nutrition researchers, Native American health care representatives and members of the Spirit Lake Tribe, Fort Totten, North Dakota, August 1998.
JR Hunt spoke on "Vegetarian versus Non-vegetarian Diets" at the 24th Annual American Agri-Women Convention, Fargo, ND, November 13, 1998.

Publications:

01. HUNT, J.R., MATTHYS, L.A. and JOHNSON, L.K. 1998. Zinc absorption, mineral balance and blood lipids in women consuming controlled lacto-ovo-vegetarian vs. omnivorous diets.... Am. J. Clin. Nutr. 67:421-430.

02. HUNT, J.R. 1997. Do common sources of dietary protein increase calcium needs? J. Am. Dietet. Assoc. 97:1370. (Letter)

03. HUNT, J.R. and JOHNSON, P.E. 1998. Zinc balance in adolescent females. Am. J. Clin. Nutr. 67:948-949. (Letter)

04. ROUGHEAD, Z.K., JOHNSON, L.K. and HUNT, J.R. 1998. Iron status and oxidative stress in rats as affected by interactions among dietary iron, zinc and copper. FASEB J. 12:A219. (Abstract)

05. HUNT, J.R. and ZITO, C.A. 1998. Iron absorption adapts to dietary iron bioavailability. FASEB J.12:A821. (Abstract)

06. HUNT, J.R. and ROUGHEAD, Z.K. 1998. Control of iron absorption in US men: a study of adaptation to dietary iron bioavailability. J. Trace Elem. Exp. Med. 11:388-389. (Abstract).

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401621 Year: 98 Project Number: 5450-51000-021-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: WHOLE BODY COUNTING AND RADIOTRACER METHODS IN RESEARCH ON MINERAL REQUIREMENTS IN HUMAN NUTRITION

Period Covered From: 02/98 To: 12/98

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

To make dietary recommendations and evaluate dietary practices that promote good mineral nutrition for the population, there must be sensitive methods for measuring mineral nutrient absorption, excretion, retention, and food bioavailability. The use of isotopic tracer methodology can effectively contribute to meeting these needs. Specifically, use of a whole body scintillation counter can safely and sensitively determine whole body retention of mineral elements that have gamma-emitting isotopes with short to moderate half-lives, such as cadmium, calcium, copper, iron, magnesium, manganese, and zinc. The whole body counting approach has the advantage of determining mineral retention without volunteer inconvenience, high variability, and incomplete sample collections associated with collecting mineral excretion data. It allows the use of a true "tracer" that does not alter the absolute mass of the mineral under investigation, and is easily and sensitively measured with minimal labor. This agreement provides the expertise of a certified health physicist to cooperate with nutrition scientists at the Grand Forks Human Nutrition Research Center, providing an interdisciplinary approach to answering nutrition questions with whole body counting methodology.

How serious is the problem? Why does it matter?

Nutrient bioavailability addresses our ability to effectively utilize the nutrients in food for body biological functions. Two diets that contain similar amounts of a nutrient, such as iron, can differ by as much as 10-fold in the amount of iron nutrient that is absorbed, retained, and utilized by the body. Internationally, zinc deficiency has been observed in humans whose diets contained considerable quantities of zinc, but that zinc was not bioavailable because of phytic acid from whole grains or legumes that interfered with absorption and lack of protein that enhances absorption. Domestically, there is concern that dietary trends (and some recommendations) to increase whole grains and legumes while reducing animal products in the diet may compromise copper, iron and zinc nutrition. The promotion of mineral supplements in the US may lead to imbalances that affect the absorption and retention of other minerals. To complement new knowledge of the importance of nutrients for optimal health, we must also know the bioavailability of and interactions among

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401621 Year: 98 Project Number: 5450-51000-021-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

nutrients from common diets, in order to provide dietary advice to the public.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition, and Intake (100%)

This research is directly related to one of ten current nutrition problems and related objectives designated in the ARS National Programs. Specifically, this research concerns the problem, "the bioavailability of nutrients in food".

What was your most significant accomplishment this past year?

Whole body counting measurements were utilized to investigate a) adaptation in iron absorption from a beef-based meal, in response to iron supplements, b) adaptation in iron absorption in response to differences in dietary iron bioavailability, and c) manganese absorption and retention, as influenced by dietary manganese and the saturation of dietary fat.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

The greater natural gamma ray background radiation at the GFHNRC was quantitatively compared with that at Lawrence Livermore National Laboratory (LLNL), Livermore, California, by recording measurements using two NaI(Tl) detectors both at GFHNRC and at LLNL. In addition to technical improvement and maintenance of the whole body scintillation counting equipment, substantial work was directed toward repair and replacement of equipment damaged by the 1997 Grand Forks flood. These efforts included production of a new Uniform Isotope Source (UNIS) board, and preliminary planning to integrate a new algorithm employing a "three-gamma ray" UNIS board into Library Least Squares programs to improve data collection independent of subject size and radioisotope distribution. DOS-based computer programs were developed for use with the repaired small animal whole body counter, and work was begun to upgrade this software to a Windows-based user friendly program. An in-depth quality control study of the GFHNRC whole body counting equipment was conducted through a student participating in the UND 1998 Research Experience for Undergraduates in Physics (REU) Program. Time-based trends showed, as expected, decreased sensitivity and increased resolution of whole body counter detectors. A large 12.5 cm x 5 cm NaI(Tl) cylindrical detector was obtained, to selectively measure cranium and other organ gamma ray emissions in metabolic studies employing radioactive tracers. Arrangements were made by the UND cooperator to receive the gamma radiation detection equipment

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401621 Year: 98 Project Number: 5450-51000-021-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

on indefinite loan from the North Dakota State Department of Health and Consolidated Laboratories. This equipment will support independent analytical identification and standardization of isotope sources. The continuous improvement in whole body counting methods provides a unique resource for extending knowledge of mineral absorption and retention. Precise whole body counting measurements allowed sensitive measurements of iron retention, revealing that men adapt iron absorption in response to changes in dietary iron bioavailability. These results demonstrated that research with short-term diets overestimates differences in iron bioavailability between chronic diets. The information can be helpful in setting recommended dietary allowances for iron.

What do you expect to accomplish during the next year?

The library least squares methodology will be further developed to establish minimal detection limits for specific radioisotopes measured against a typical K-40 background, and to utilize the new algorithm based on the "three-gamma ray" UNIS board. This work will aid data analysis and interpretation of the variation caused by body size and density in self-absorption of internally produced radiation. Improved precision of whole body counting will result, especially for persons with varying body size and shape, and whole body counting will be more useful in determining changes in body composition associated with diet and/or exercise over time. Opportunity to work on this project will be offered as a Research Experience for Undergraduates in Physics (REU) project during the summer of 1999. In addition, support will be provided for the acquisition, standardization, and use of Ca-47, no longer available commercially.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

Because of the uniqueness of the whole body counting equipment and technology, the major transfer of technologies from the whole body counter work have occurred through reports and publications of the results of the major nutrition projects which employed the counter, and are reported under those specific CRIS reports. Application information was shared through discussions with Medical Physicists at Brookhaven National Laboratory and with personnel at Lawrence Livermore National Laboratory.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Glenn Lykken gave a technical presentation about findings on radon daughters in relation to Alzheimer's and Parkinson's diseases at the Experimental Biology '98 meeting in San Francisco, California.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401621 Year: 98 Project Number: 5450-51000-021-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Publications:

01. MOMCILOVIC B., et al. 1997. What do radon daughters tell us about Alzheimer's disease, Parkinson's...Cl- ion membrane transport, pp. 574-588. IN: Mengen und Spuren Elemente; 17. Verlag Harald Schubert, Leipzig.

02. MOMCILOVIC, B., ALKHATIB, H.A., et al. 1998. Radon daughters in Alzheimer's disease and Parkinson's disease: Beyond the tangles and substantia nigra...and Cl- ion membrane transport. FASEB J.12:A752.

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401160 Year: 98 Project Number: 5450-51000-021-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s):

Title: ADAPTATION IN THE ABSORPTION OF IRON FROM BEEF

Period Covered From: 01/98 To: 12/98

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

Concerns about high iron stores and the risk of chronic diseases such as heart disease and cancer have led to speculation that red meat intake should be limited because it is an excellent source of highly absorbable iron. There is special concern that heme iron absorption is not biologically controlled to the same degree as nonheme iron absorption. This project is related to CRIS 5450-51000-021-00D by the same investigators, and is limited to a single human experiment that has been partially funded by the National Cattlemen's Beef Association. This experiment will determine a) whether heme iron absorption from a meat-based meal is reduced after iron supplementation, b) whether nonheme iron absorption from a meat-based meal is reduced after iron supplementation, c) whether intestinal ferritin production, as measured in fecal samples, is increased after iron supplementation, and is associated with changes in heme and nonheme iron absorption, and d) whether serum ferritin is increased substantially after iron supplementation, and if any increase in serum ferritin persists after iron supplementation is discontinued.

How serious is the problem? Why does it matter?

Iron nutrition is a double-edged sword. While adequate iron nutriture in infants, children, and childbearing age women is essential for cognitive development and work capacity, excessive body iron in men and postmenopausal women is hypothesized to increase oxidative stress and the risk of chronic diseases such as heart disease and cancer. Research on dietary iron bioavailability provides information for policy decisions about recommended dietary allowances, nutrient supplementation, food enrichment and fortification standards, and dietary guidelines for the public. Results from this research may suggest that red meat is a good source of iron for persons with inadequate iron nutriture, without providing excessive iron for those with adequate iron stores.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition, and Intake (100%)

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401160 Year: 98 Project Number: 5450-51000-021-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s):

This research is directly related to one of ten current nutrition problems and related objectives designated in the ARS National Programs. Specifically, this research concerns the problem, "the bioavailability of nutrients in food".

What was your most significant accomplishment this past year?

Determined that daily iron supplementation reduces the efficiency of nonheme, but not heme iron absorption from food. The absorption of nonheme iron from food decreased significantly ($p<0.001$) by about 36% after 12 wk of iron supplementation. A slight reduction in heme iron absorption with time in the study was not significantly related to the supplementation treatment.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

The above finding that nonheme, but not heme iron absorption adapts in response to daily iron supplementation will be useful in assessing the ability of humans to adapt to differences in the quantity and bioavailability of dietary iron. These were the first findings from this project that is expected to conclude in 1999.

What do you expect to accomplish during the next year?

Further work on this study will determine the influence of iron supplementation and subsequent time after iron supplementation on iron stores, as indicated by serum ferritin, and on intestinal adaptation, as indicated by fecal ferritin. Cross-sectional analysis will also indicate the association, if any, between heme iron absorption and body iron stores. If heme iron absorption is unrelated to either iron supplementation or to body iron stores, it will suggest little or no physiological control of heme iron absorption. Final statistical analysis and reporting to the National Cattlemen's Beef Association is scheduled for the spring of 1999. This will include preparation and submission for publication of a technical scientific paper.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

The initial iron absorption results have been submitted in abstract form to the organizers of the Experimental Biology '99 meeting and to the National Cattlemen's Beef Association. A technical publication will be prepared, and shared with the National Cattlemen's Beef Association, when data analysis is completed in 1999.

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Accession: 0401160 Year: 98 Project Number: 5450-51000-021-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s):

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

JR Hunt spoke on "Vitamins and Minerals: To Supplement or Not to Supplement?" North Dakota State University Extension Service, interactive broadcast from Grand Forks to 8 sites in ND, March 3, 1998.
JR Hunt spoke on "Human Research with Zinc and Iron" South Dakota State University's 15th Annual Nutrition Seminar, Brookings, SD, March 24, 1998.
ZK Roughead spoke on "What is New in Iron Research at Grand Forks Human Nutrition Research Center. North Dakota Dietetic Association, Spring Convention, May 13, 1998, Bismarck, North Dakota.

Publications:

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400524 Year: 98 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: HUMAN MINERAL ELEMENT REQUIREMENTS AND THEIR MODIFICATION BY STRESSORS

Period Covered From: 05/96 To: 05/01

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

Dietary excesses, deficiencies and imbalances contribute to the susceptibility and severity of a number of chronic diseases of major health and economic consequence; these include coronary heart disease, some cancers, hypertension, stroke, diabetes mellitus, atherosclerosis, cataracts and osteoporosis. Subnormal mineral element nutrition has been implicated in all of these chronic diseases. Furthermore, there is evidence that mineral elements are most important in regards to maintaining health, or will produce pathology most markedly with low intakes when a nutritional, metabolic, hormonal or physiological stressor is present that enhances the need, or interferes with the utilization of the elements. However, the importance of mineral element nutrition in the promotion of health and disease prevention, and in the reduction of health care costs, and factors that affect this importance has not been fully defined. This includes the need to provide research findings to ascertain the validity of claims that magnesium is of practical concern for maintaining bone and cardiovascular health, especially when diets are low in copper and high in foods or drinks that provide high amounts of fructose; that high dietary zinc adversely affects blood lipid profiles including the amount of good (HDL) and bad (LDL) forms of cholesterol presumably through altering copper metabolism; that boron, copper, zinc, and manganese status affects calcium utilization and metabolism and thus the susceptibility to osteoporosis; and that copper, manganese, selenium and zinc affect the susceptibility to cancer induced by xenobiotic substances and reactive oxygen species.
Studies with human volunteers are and will be conducted. This includes studies examining the effects of varying intakes of zinc at different intakes of dietary copper on lipid profiles, bone status indicators, and on reactive oxygen species metabolism; the determination whether low magnesium intakes in combination with stressors that apparently increase its need induces a neurogenic inflammatory response leading to oxidative damage that can lead to pathophysiology such as cardiomyopathy, migraine headaches, and abnormal central nervous system function; and whether boron supplementation of individuals with suspected low boron status improves cognitive and motor function and indicators of bone health. Also, the effect of various intakes of mineral elements, especially copper and selenium, on the cellular functions that might be indicative of cancer susceptibility will be determined in terminally differentiated colonic epithelial cells recovered from freshly passed human stools.

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0400524 Year: 98 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107. 100%

How serious is the problem? Why does it matter?

Dietary factors, including trace element nutriture, are associated with 5 of the 10 leading causes of death, including coronary heart disease, certain types of cancer, stroke and atherosclerosis. An example of the importance of nutrition in chronic disease is colon cancer, which is the second leading cause of cancer mortality in the United States and the fourth most common cause of cancer mortality worldwide. It is believed that diet is the single greatest contributor to human cancer, possibly accounting for 35 to 45% of the disease. Dietary excesses, deficiencies and imbalances in trace mineral intake is one factor that can affect cancer susceptibility.
Among those diseases that are linked strongly to diet, the cost for treatment and care in the United States exceeds $200 billion per year. Among the diseases associated with subnormal mineral element nutrition, the annual economic costs is estimated to be greater than $80 billion for cardiovascular disease, and $10 billion for osteoporosis. Several mineral elements associated with these chronic diseases including boron, copper, and magnesium have been shown to be routinely low in diets in the United States. Thus, providing information about requirements and factors that affect those requirements of critical mineral elements should result in policies and programs that improve intakes of these nutrients that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care expenditures.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research relates to the National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The research is related to the specific National Program Components of 1) Changes in needs for nutrients throughout the life cycle. 2) Function and metabolism of nutrients for cognitive and physical development. 3) Definition of marginal deficiencies or borderline deficiencies. 4) Nutritional needs of a diverse population. The research will develop information about the effects of deficiency or imbalance of specific nutrients on biochemical, physiological and psychological functions to facilitate their detection and prevention, and to define requirements for health and well-being. It also will establish safe and optimal intakes, and the roles in risk or prevention of diet-related disorders, of mineral elements.

What was your most significant accomplishment this past year?

High dietary zinc significantly increased plasma zinc concentrations, immunoreactive ceruloplasmin concentrations, platelet cytochrome C oxidase activity, extracellular superoxide dismutase activity, white blood cell

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0400524 Year: 98 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

5'-nucleotidase activity and bone specific alkaline phosphatase activity. The findings with extracellular superoxide dismutase activity and white blood cell 5'-nucleotidase activity suggests that these may be good indicators for the assessment zinc status in humans. The lack of a marked adverse effect of high dietary zinc on indicators of copper status even when dietary copper is 1 mg per day suggests that intakes of 53 mg zinc per day does not have a marked adverse effect on copper metabolism.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

Experimental evidence using human volunteers shows that low dietary intakes of magnesium induces changes in indices used to assess the susceptibility to cardiovascular and calcium metabolism disorders. The low magnesium intake induced heart rhythm abnormalities, altered cardiovascular function and energy metabolism in postmenopausal women. Because magnesium is often consumed in inadequate amounts according to dietary surveys, these findings indicate that magnesium is of practical nutritional and clinical importance in the prevention of heart disease. Calcium balance can be maintained in postmenopausal women with intakes less than 800 mg/day, but can be altered by undesirable changes in dietary magnesium, fructose and copper. These findings show that high dietary intakes of calcium are not the complete answer to the prevention of bone loss leading to osteoporosis. Intakes of nutrients such as magnesium, boron, copper and fructose must be appropriate for maintaining healthy bones. That is, if these nutrients are consumed in appropriate amounts, high dietary calcium intakes which are difficult to achieve by diet alone are unnecessary to prevent bone loss in postmenopausal women.
The consumption of high fructose decreases calcium balance with the effect more marked when dietary magnesium is low. This finding indicates that the consumption of high amounts of carbonated beverages sweetened with high fructose corn syrup is detrimental to the formation and maintenance of healthy strong bones.
The frequency of xenobiotic-induced preneoplastic lesions associated with colon cancer (aberrant crypt foci) development was significantly increased in animals fed low dietary copper and tended to be increased in animals fed low dietary manganese and high dietary iron. Altered activities of antioxidant enzymes, known as superoxide dismutases, were significantly correlated with the number of the anatomical lesions associated with colon cancer. These findings suggest that dietary alterations which affect superoxide dismutase activity affect cancer susceptibility. Furthermore, the effect of dietary copper and manganese on aberrant crypt foci formation may have practical implications because diets in the United States often contain copper and manganese in amounts less than their estimated safe and adequate daily dietary intakes.
High dietary zinc significantly decreased plasma cholesterol and lipoprotein A concentrations, and significantly increased various

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0400524 Year: 98 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

indicators of copper status including platelet cytochrome C oxidase activity and ceruloplasmin concentrations. These findings do not support the dogma that high dietary zinc decreases the retention of copper from the diet, or adversely affects copper metabolism, which results in adverse effects on blood lipid profiles including the amount of good (HDL) and bad (LDL) forms of cholesterol.

What do you expect to accomplish during the next year?

Methods will be developed for the use of isolated colonocytes to assess the effects of changes in mineral element intakes on cancer susceptibility of humans. Human volunteers will be fed specific intakes of certain mineral elements and exfoliated cells will be prepared from their fecal material. Functions of these cells that might be indicative of cancer susceptibility in the whole individual will be determined.
A human experiment will be initiated that will examine whether low magnesium status, especially with low copper or high fructose intakes, induces changes in the neurogenic inflammatory response which could be indicative of being vulnerable to oxidative damage that can lead to pathophysiological consequences in heart and central nervous system function.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

Information about nutritional and clinical importance of the mineral elements magnesium, copper, boron, zinc, and manganese as it becomes available is routinely transferred to a variety of customers. The customers include the public through web pages of professional organizations and via the popular media, and other scientists through presentations at national and international meetings and professional publications.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

"Nutrition and Osteoporosis," a presentation by F.H. Nielsen for the Interactive Video Network Program of the North Dakota State University Continuing Education Program.
"Magnesium - A Mineral Nutrient of Concern for Optimal Bone, Brain, and Cardiovascular Function." Presentation given by F.H. Nielsen at the Eighth Medical Symposium: Research Day at the Ross University School of Medicine, Portsmouth, Dominica.
"The Minefield of Mineral Supplements - Contributors to Health or Just a Source of Money for the Mountebank?" Presentation given by F.H. Nielsen

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Accession: 0400524 Year: 98 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

as part of the Staff Training in Extramural Programs at the National Institutes of Health, Bethesda, MD.

Publications:

01. WHO Task Group On Environmental Health Criteria for Boron (included F.H. Nielsen). 1998. Environmental Health Criteria 204: Boron. World Health Organization, Geneva, Switzerland, pp. 201.

02. NIELSEN, F.H. 1998. The importance of making dietary recommendations for elements designated as nutritionally beneficial, pharmacologically beneficial, or conditionally essential. J. Trace Elem. Exp. Med. 11:438.

03. NIELSEN, F.H. 1998. Boron supplementation of perimenopausal women affects boron metabolism, and indices associated with macromineral metabolism, hormonal status and immune function. J. Trace Elem. Exp. Med. 11:437-438.

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400048 Year: 98 Project Number: 5450-51000-022-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.3.1.2 50%
National Program(s): 107 100%

Title: NEW METHODS OF ASSESSMENT OF SPECIFIC SUBOPTIMAL MINERAL NUTRIENT STATUS IN HUMANS

Period Covered From: 01/98 To: 12/98

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

The essentiality of zinc for human health and well being is well established and the consequences of severe zinc deficiency have been documented in several populations worldwide. Studies in animals and people have shown that zinc is involved in many body processes including the synthesis of protein, cellular growth and repair, regulation of various hormones, the function of the immune system, regulation of appetite and the regulation of blood pressure. Zinc deficiency can result in impaired growth, delayed sexual maturation, impaired wound healing, loss of taste and smell acuity, impaired immune function and dermatitis. Conversely, chronic excess intakes of zinc is capable of interfering with the uptake and metabolism of other trace elements, notably iron and copper; prolonged ingestion of excessive amounts of zinc can result in conditions such as anemia, elevation of cholesterol, and lower activity of superoxide dismutase, a copper and zinc containing enzyme. However, studies designed to examine the consequences of mild deficiency or excess zinc intake and to establish an optimal range of intakes have been impaired because methods currently available for the assessment of zinc status in humans are unsatisfactory. Furthermore, there is little known about the consequences of copper supplements during periods of low zinc intake.
These issues are being resolved in a human metabolic study examining the effects of varying intakes of zinc at different intakes of dietary copper. In addition, newer proposed indicators of zinc status are being evaluated. Postmenopausal women are being studied because of their susceptibility to osteoporosis (both copper and zinc are involved with bone metabolism), concerns about possible mild zinc deficiency in this population, and concerns regarding their use of excessive zinc supplements.

How serious is the problem? Why does it matter?

Diagnosis of zinc deficiency in humans is difficult because the condition produces a range of nonspecific clinical symptoms. Chronic zinc deficiency may be indicated by impaired immune function and reduced rate and/or quality of growth in children. Although zinc deficiency is believed to not be common in the United States, it is relatively common in the Middle East where people eat diets consisting mainly of unrefined

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400048 Year: 98 Project Number: 5450-51000-022-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.3.1.2 50%
National Program(s): 107 100%

cereals and little animal protein. However, recent studies have suggested that mild zinc deficiency may be a significant clinical problem in elderly people living at home in the United States. In view of the many roles of zinc in human health, it is important that optimal zinc status be maintained. However, lacking a reliable indicator of human zinc deficiency and obvious clinical features, the occurrence of mild zinc deficiency or its significance to human health is uncertain.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research relates to the National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The research is related to the specific National Program Components of 1) Definition of marginal deficiencies or borderline deficiencies. 2) Bioavailability of nutrients in foods. This research will develop new indicators of nutritional status which can be used to define borderline deficiencies and to define requirements for health and well-being.

What was your most significant accomplishment this past year?

High dietary zinc significantly increased plasma zinc concentrations, extracellular superoxide dismutase activity, white blood cell 5'-nucleotidase activity and bone specific alkaline phosphatase activity. These findings suggest that extracellular superoxide dismutase, white blood cell 5'-nucleotidase and bone specific alkaline phosphatase activities may be good indicators for the assessment on zinc status in humans.
In contrast, other potential indicators of zinc status were found to be unresponsive to changes in dietary zinc. These include amyloid precursor protein expression, red blood cell membrane 5'-nucleotidase activity, red blood cell membrane alkaline phosphatase activity and metallothionein concentrations.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

Because abnormalities of both zinc and copper metabolism have been implicated in the development of Alzheimer's disease which is characterized by a gradual loss of memory, reasoning and judgement, a study was conducted using laboratory rats as a model to determine whether changes in dietary zinc and/or copper concentrations might affect the amount of a substance that accumulates in the brain of Alzheimer's disease patients and whether this substance could be used as an indicator of copper and zinc status. Neither changes in dietary zinc nor dietary copper affected the amount of the substance studied; this finding should be useful to research scientists and medical doctors attempting to

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Accession: 0400048 Year: 98 Project Number: 5450-51000-022-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.3.1.2 50%
National Program(s): 107 100%

determine the cause of Alzheimer's disease.

What do you expect to accomplish during the next year?

The analysis from the study will be completed and the results will be written and submitted to scientific journals.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

Information about nutritional and clinical importance of zinc as it becomes available is routinely transferred to a variety of customers. The information has been sent to the International Lead Zinc Research Organization (ILZRO) via informal communications and we are in the process of writing a final report that will be available in March. The information has been available to other scientists through presentations at national meetings and we are currently in the process of writing professional communications.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

CD Davis spoke on "Extracellular superoxide dismutase activity: a promising indicator of zinc status in humans" at the Experimental Biology 98 meeting.

Publications:
01. DAVIS, C.D., KLEVAY, L.M., MILNE, D.B. and NIELSEN, F.H. 1998. Extracellular superoxide dismutase activity: a promising indicator of zinc status in humans. FASEB J. 12: A346.

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149894 Year: 98 Project Number: 5450-51530-004-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 25% 5.3.1.2 75%
National Program(s): 107 100%

Title: DEVELOPMENT AND EVALUATION OF METHODS FOR THE CLINICAL EVALUATION OF MINERAL NUTRITIONAL STATUS

Period Covered From: 05/96 To: 05/01

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

Although the essentiality of many trace elements such as copper and zinc is well established, there are currently no adequate and generally agreed upon clinical tests for evaluating copper, zinc, and magnesium nutritional status in humans. The lack of accurate diagnostic tests has impeded programs for determining human nutritional requirements and diagnosis of marginal trace mineral deficiencies that may play roles in the etiology of some chronic diseases, such as ischemic heart disease, osteoporosis, and adult onset diabetes.

Methods for the evaluation of copper, zinc, and magnesium nutritional status are being developed, and reference ranges are being established and critically evaluated for usefulness in studies using human volunteers.

How serious is the problem? Why does it matter?

Food consumption surveys have suggested that significant portions of the population have intakes of copper, zinc, or magnesium that are below the amount needed for maintaining good health. Yet wide-spread deficiencies of these nutrients are unrecognized, mainly because of the lack of clear-cut methods for detecting marginal deficiencies. Development of specific, accurate, and cost-effective tests for the measurement of nutritional status will aid clinicians in detecting nutritional deficiencies and imbalances in their early stages. This is particularly important for potentially malnourished portions of the population such as children and the elderly. Early detection of defects in trace element nutrition can result in considerable savings in costs in treating chronic symptoms. Additionally, techniques developed will be applicable to studies on the human requirements and metabolism of trace elements. Knowledge gained by these studies will enable the evaluation of federal food and nutrition programs, and administration of programs that contribute to the health and well-being of people.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

Research on human nutrition requirements, food composition and intake is one of the priority ARS National Programs, number 107. In order to carry

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National Program(s): 107 100%

out components of National Programs such as 'Definition of marginal deficiencies or borderline deficiencies' and 'What are effective intervention strategies, specific, reliable and cost-effective methods for assessing nutritional status are required. This need is recognized in a program component that states "Develop efficient economical methods of measuring the amount of essential nutrients consumed and the biologically active substances in human tissues and fluids to better assess the nutritional status of individuals."

What was your most significant accomplishment this past year?

A metabolic unit study that investigated the effects of low and moderately high zinc intakes on zinc, copper, and iron status indicators helped define several potential sensitive indicators of zinc nutritional status. Zinc indicators that were the most sensitive to changes in zinc status, based on increases in values when zinc supplements were fed, include plasma zinc, extracellular superoxide dismutase, erythrocyte membrane 5' nucleotidase, and bone specific alkaline phosphatase.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

A study investigating the interaction between dietary copper and magnesium demonstrated that serum and ultrafilterable serum magnesium were directly related to dietary magnesium; the changes were greater in the subjects who were fed a marginal copper diet than those supplemented with copper. A significant interaction between copper and magnesium affected the copper-containing enzyme superoxide dismutase in red blood cells (ESOD). ESOD was higher in the copper supplemented subjects when 100 mg magnesium per day was fed but it was not affected by copper when 300 mg magnesium per day was fed. The sequence in which magnesium supplements were given obscured the magnesium effects on variables such as serum magnesium, serum ionized magnesium, cholesterol, glucose and red cell superoxide dismutase; changes were greatest when placebo was fed first and were smaller or lacking when magnesium was fed first. This suggests that prior high magnesium inhibits or delays the appearance of magnesium deprivation signs. This information is of importance for developing further studies on magnesium nutritional requirements and on the effects of magnesium deprivation.
A study investigating the interaction between dietary fructose and dietary magnesium showed that high dietary fructose significantly increased magnesium balance during both low and high magnesium intakes. Serum ultrafilterable and ionized magnesium were also affected by fructose and magnesium intakes; they were higher when fructose was fed and when magnesium intakes were high. High dietary fructose also depressed both calcium and phosphorous balances and increased urinary loss of phosphorous. These findings suggest that high dietary fructose adversely affects calcium and phosphorous metabolism in humans, particularly when dietary magnesium

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Mode Code: 5450-10-00 STP Codes: 5.1.3.3 25% 5.3.1.2 75%
National Program(s): 107 100%

is low. This is of concern, since recent surveys indicate increased consumption of fructose-based sweeteners and that a significant portion of the population is consuming diets low in magnesium. Further studies are indicated to see if a high fructose diet coupled with low dietary magnesium and marginal calcium leads to bone loss.
Plasma zinc, extracellular superoxide dismutase, erythrocyte membrane 5' nucleotidase, and bone specific alkaline phosphatase were shown to be sensitive indicators of zinc nutritional status. This knowledge will help in designing studies defining the human requirements of zinc during different life stages and for surveys evaluating the zinc status of populations at risk of chronic diseases where zinc may play a role.
An interaction between zinc and copper in postmenopausal women affected several zinc, copper, and iron responsive variables. The interaction between zinc and copper affected extracellular superoxide dismutase activity; the increase in activity between low zinc and high zinc was greatest in the low copper group. In addition, dietary copper seemed to increase the activity when 3 mg zinc/day were fed, but not when 53 mg zinc/day was fed. Copper and iron indicators that were depressed slightly when zinc supplements were fed included erythrocyte copper-zinc superoxide dismutase, ceruloplasmin specific activity (enzyme activity/immunoreactive protein), and hemoglobin. The depressions by zinc seemed greatest when one mg copper per day was fed. Other copper and iron indicators were either not affected or increased when zinc was supplemented. This may have been the result of decreased protein synthesis when 3 mg zinc/day was fed. Thus, no marked adverse effect of 53 mg of zinc per day in the diet on copper and iron nutriture could be demonstrated. Thus, zinc intake of this magnitude may not be as detrimental as feared on copper metabolism if copper intake is close to adequate.

What do you expect to accomplish during the next year?

Population-based reference ranges will be established for many of the more newly defined indicators of copper and zinc nutritional status. Men and women whose blood cell copper-containing enzymes are in the lower 20 percentile of the population will be supplemented with copper or a placebo to determine the sensitivity of these indicators in predicting low copper status and to determine if marginal copper deficiency is a problem in this population.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

1. Consultant on a study "Copper, zinc, manganese and bone health in postmenopausal women being conducted at the University of Georgia. Provided methods relating to the assessment of copper, zinc, and manganese and provided advice on experimental design and best methods to use.

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National Program(s): 107 100%

2. Provided methods on blood cell separations and analytical procedures developed in this laboratory to other ARS scientists and investigators in other laboratories around the world.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

1. Presentation: "Adult responses to various short-term dietary copper intakes: Insights on human copper requirements and indicators of status" as 'distinguished visiting scientist at the University of Georgia.
2. Work on magnesium- fructose interaction effects on bone minerals covered in popular press, such as Food Product Design Magazine and on the USDA web page Science for Kids.
3. Study and volunteers participating in the study on the interaction between zinc and copper was featured in the Grand Forks Hearld.

Publications:

01. MILNE, D.B. 1998. Copper intake and assessment of copper status. Am. J. Clin. Nutr. 67:1041S-45S.

02. MARCHELLO, M.J., SLANGER, W.D., HADLEY, M., MILNE, D.B. and DRISKELL, J.A. 1998. Nutrient composition of bison fed concentrate diets. J. Food Comp. Anal. 11:231-9.

03. MILNE, D.B. 1998. Trace Elements, pp. 1029-1055. IN: C.A. Burtis and E.R. Ashwood (eds.) TIETZ Textbook of Clinical Chemistry, 3rd Ed. W.B. Saunders, Philadelphia, PA.

04. MILNE, D.B. and NIELSEN, F.H. 1998. Dietary magnesium and fructose affect macromineral metabolism in men. FASEB J. 12:A508.

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

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MINERAL NUTRIENT REQUIREMENTS

MANAGEMENT UNIT

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400186 Year: 98 Project Number: 5450-51000-018-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

Title: DIETARY TRACE ELEMENTS AND PHYSIOLOGY OF THE CARDIOVASCULAR AND RELATED SYSTEMS

Period Covered From: 02/96 To: 02/01

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

The cardiovascular system (heart and circulation) is a target for biochemical deficit and structural damage in dietary copper deficiency. Although progress is being made in identifying defects in cardiovascular physiology in copper deficiency, the bases or mechanisms for these functional changes are not completely clear. The known functional changes are not related in a clear fashion to the known morphological and biochemical changes. Additionally, because most of the studies on trace element deficiencies, in particular that of copper, have not been done in humans or with dietary trace element intakes consistent with human consumption, the relationship of such findings to human health is not clear. The approaches to resolving this problem include:
A. Determination of functional changes in blood vessels and, in particular, clarification of changes in signal transduction pathways in smooth muscle and endothelium with copper deficiency. Relevant studies will be performed with isolated vessels, on isolated organs and on whole animals. The ultimate goal is to determine the contribution of adequate copper nutrition to maintenance of blood flow to organs and to maintenance of blood pressure.
B. Identification of functional changes in the heart and their relationship to metabolic and biochemical alterations in trace element (copper) deficiencies. The focus will be to determine coronary blood vessel and cardiac muscle vulnerability to physiologic and metabolic stressors including, but not limited to, adrenaline stimulation and simulated heart attack (cessation and re-starting of blood flow to the heart). Isolated heart and whole animal models will be used.
C. Elucidation of general biochemical mechanisms of damage in copper deficiency. Oxidative stress continues to be a strong, although somewhat equivocal, candidate as a mechanism for generalized damage. Damage by oxidative mechanisms will be compared with that caused by another mechanism, glycation. The aim is to attempt to relate known enzymatic, metabolic and hormonal changes to the deterioration of function that occurs in copper deficiency. Various organs will be tested, but the primary focus will be on the heart and blood.

How serious is the problem? Why does it matter?

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Accession: 0400186 Year: 98 Project Number: 5450-51000-018-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

Prior studies have indicated that dietary copper deficiency has considerable potential for contributing to chronic disease (for example, ischemic heart disease, atherosclerosis, high blood pressure) and the debilitating effects of aging. Experimental evidence indicates that a third or more of the American population may be consuming less than the Estimated Safe and Adequate Dietary Intake of copper.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

These studies directly contribute to National Program 107, Human Nutrition Requirements, Food Composition, and Intake. By examining the effect of reduced dietary copper intake on biochemical relationships in the heart and blood vessels, the subsequent effects those changes have on heart and blood vessel function and the parallels those changes have to diseases such as diabetes, aging and heart disease, these studies directly address the specific Program Components "Studies of trace element nutrition -- Biochemical and health consequences of suboptimal trace element intakes" and "Evaluations of the role of diet in affecting risk factors for disease in humans -- Abilities of dietary trace elements to reduce risk of cardiovascular disease."

What was your most significant accomplishment this past year?

Strong evidence was found supporting the view that glycation, the undesirable binding of sugar to proteins, is enhanced in dietary copper deficiency. Blood analysis revealed the presence of glycated hemoglobin and fructosamine (blood proteins with sugar bound to them) as well as pentosidine (a product of blood protein damaged by glycation). Because glycation is a process that is increased in diabetes and aging, the present finding suggests that reduced copper intake may worsen the consequences of these two conditions.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

Elevations in concentration of two important chemical messengers, nitric oxide and cyclic GMP, in copper-deficient hearts suggested their possible role in reduction of heart contractile force in copper deficiency. These and subsequent studies (some planned for next year) will help to define the molecular basis for altered heart function when dietary copper is restricted.
Measurements of heart and blood vessel function in copper deficient animals helped to show that, although cardiac output was not altered by copper deficiency, blood vessel resistance was reduced and volume of blood ejected per beat (stroke volume) of the heart was elevated; the higher stroke volume may contribute to the pathologically greater size of copper-

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National Program(s): 107 100%

deficient hearts. These and succeeding physiological measurements will help to characterize heart function in dietary copper deficiency.
Studies with collaborators have continued to examine the effect of copper deficiency on blood clotting function. The most recent findings have shown that the aggregation of blood platelets to one another was increased and that adhesion of platelets to blood vessel endothelial cells was reduced. Further, these findings were associated with an alteration of two platelet clotting factors, fibrinogen and von Willebrand factor. These studies emphasize the importance of dietary copper to prevent bleeding.
Another collaborative study found that the dilation of blood vessels in response to an inflammatory agent was exaggerated in copper-deficient rats. By use of appropriate blocking agents, the potential mechanism(s)responsible for this change were delineated. This study shows the importance of proper copper intake in mediating the body's inflammatory response to injury.

What do you expect to accomplish during the next year?

Determine whether and to what extent the nitric oxide signal transduction pathway is involved in the altered mitochondrial respiration of copper-deficient hearts. This will help to clarify the mechanism whereby copper deficiency depresses heart function.
Determine whether a direct association can be made between formation of advanced glycation end-products and altered heart function of copper deficiency.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

Findings of the described studies have been disseminated to the general public through an article written for the nutrition section of the local newspaper and by information provided on the laboratory's internet website. Presentations describing the findings have been made to scientists at a national meeting.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Article in local newspaper (Grand Forks Herald) entitled "Copper? You Bet Your Heart", February, 1998.

Publications:
01. SAARI, J.T. and DAHLEN, G.M. 1998. Nitric oxide and cyclic GMP are elevated in hearts of copper-deficient rats. Med. Sci. Res. 26:495-497.

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National Program(s): 107 100%

Publications: (Continued)

02. SAARI, J.T. and DAHLEN, G.M. 1998. Nitric oxide synthase inhibition ameliorates the anemia of dietary copper deficiency. FASEB J. 12:A242. (Abstract)

03. SCHUSCHKE, D.A., LOMINADZE, D., SAARI, J.T. et al. 1998. Von Willebrand Factor (vWF) but not superoxide dismutase (SOD) restores attenuated platelet thrombosis in copper-deficient rats. FASEB J. 12:A241. (Abstract)

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400106 Year: 98 Project Number: 5450-51000-019-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 90% 5.1.4.3 10%
National Program(s): 107 100%

Title: MINERAL ELEMENT NUTRITION, NEUROPSYCHOLOGICAL FUNCTION AND BEHAVIOR

Period Covered From: 03/96 To: 03/01

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

Behavior is unique as a criterion for establishing nutritional adequacy because it represents the functional integration of all biological systems, including compensatory mechanisms that often determine the practical importance of a nutritional deficit or excess. However, increased knowledge and a better understanding of the relationships among behavior, neuropsychological function and mineral element nutrition is required before making recommendations for mineral intakes that will facilitate optimal neuropsychological health and performance. Neuropsychological and behavioral consequences of mild and moderate deficiencies in biologically essential mineral elements are determined with the goal of improving health, work and school performance, and sense of well-being in the population. Specifically, studies are designed to determine: the role of mineral elements in cognition (i.e., attention, perception, learning, memory and reasoning) and spatial and motor skills; the effect of mineral nutrition on mood states and emotional and social adjustment; the impact on nutrition-behavior relationships of potential mediating factors, including environmental and endogenous stressors like noise, temperature, sleep duration and quality, and menstrual and menopausal symptoms; and, the effect of mineral nutrition on electro-physiology indexing brain function to gain insights into the mechanisms for nutritional effects on performance and sense of well-being. New methods and technologies are developed to increase efficacy of behavioral assessments and promote their use by other nutrition scientists. Studies of healthy adults and children are complemented by animal studies.

How serious is the problem? Why does it matter?

Findings obtained during the past 40 years indicate that the mineral elements boron, copper, iodine, iron, magnesium, manganese, selenium and zinc likely are important for normal neuropsychological function and behavior of adults and children. However, previous studies have yet to establish the reliability of effects of graded mineral intakes on behavior or to adequately characterize the relationship between mineral element nutrition and brain function and cognition. Such information is critical to characterizing the mechanisms responsible and is needed to apply findings to real-world problems. To respond to public interest in the

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National Program(s): 107 100%

relationship between nutrition and performance, and potentially to improve public health, productivity and sense of well-being, there is a great need to increase our knowledge of the functional consequences of graded intakes of mineral elements, and especially the consequences of marginal intakes common in many segments of the population. Food consumption surveys indicate that intakes of calcium, copper, iron, magnesium and zinc are significantly below the RDA or ESADDI for large segments of the adult population in the United States and worldwide, and many reviews have concluded that mild-to-marginal deficiencies in these and other mineral elements are particularly likely in the groups targeted by this research (e.g., women, children, elderly). Further, increased knowledge of the relationship between mineral element nutrition, neuropsychological function and behavior is needed for a more complete determination of nutrient requirements, establishing recommended dietary intakes, and evaluating the efficacy and adverse effects of taking dietary supplements, a multi-billion dollar industry in the United States.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%)
The determination of dietary requirements for optimal cognitive function and performance has been identified as a national need. This research furthers ARS objectives by directly evaluating, under highly controlled conditions, the effects of mineral element nutrition on neuropsychological function and behavior of adults, including the elderly, and in children and adolescents. Examining the combined effects of nutritional insults and exogenous and endogenous stressors offers insights into ways to improve performance in work and school, and in other situations with a high demand. Understanding the true role of mineral element nutrition in neuropsychological function and behavior also helps individuals and groups to more knowledgeably evaluate nutrition claims, and promotes healthy and cost-efficient dietary selections.

What was your most significant accomplishment this past year?

Zinc (Zn) is essential for growth and early development, but the relationship between Zn and cognition in later development is largely unknown. Research this year determined that short-term supplementation with zinc (20 mg/day) combined with other micronutrients (50% recommended daily intakes) may improve some aspects of cognitive function of school-aged Mexican-American children, who are at increased risk for zinc deficiency primarily because of high intakes of dietary phytate. This research complements earlier work by this laboratory that found a similar treatment regimen improved cognitive and psychomotor function of Chinese

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National Program(s): 107 100%

children with a high incidence of zinc deficiency primarily due to inadequate Zn intakes. Findings provide further indication that zinc supplementation may significantly benefit cognitive function in deficient populations, and eventually may be used in setting recommended intakes for Zn and formulating school meal programs.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

Determined that zinc (Zn) and micronutrient supplements improved cognitive and psychomotor function of rural and urban Chinese school children. Motor skills, including manual dexterity and eye-hand coordination, visual perception, memory for simple shapes and complex objects, and reasoning were functions most affected by Zn (20 mg/day) and Zn combined with other micronutrients at 50% recommended intakes. Findings indicate that cognitive and psychomotor function, and thus school performance, may be suboptimal in the >25% of Chinese children and 6-10% of school-aged children in the United States who are Zn deficient. This was the first evidence that zinc supplementation of young children may improve cognition and psychomotor function. Findings were widely reported by the national media, followed by many inquiries from the public, private industry and granting agencies, and by invitations to speak on this research. This research led directly to collaborative studies of cognitive and psychomotor effects of zinc supplementation of Mexican-American school children (supported by grants from the Gerber Foundation and USDA National Research Initiative), of zinc and iron supplementation of young women (supported by grant from the US Army Medical Research, Development, Acquisitions and Logistics Command), and of zinc deprivation of young adult men (supported by USDA ARS Western Human Nutrition Research Center).
Manganese (Mn) is essential for normal brain function and behavior, and there has been speculation that moderate Mn intoxication or deficiency may be associated with increases in aggressive behavior. Experimentally determined that adult male rats fed diets deficient (1 ug/g diet) or excessive (100 ug/g diet) in Mn were generally less active than those fed adequate Mn (10 ug/g diet), and consequently, these rats engaged in fewer aggressive behaviors (attacking, biting, wrestling, aggressive contact) and displacement activities (exploration, self-grooming), and more posturing. However, rats fed diets high in Mn but low in calcium (Ca; 2500 versus 5000 ug/g diet) did show increased aggressive behavior compared to rats fed other diets. Findings provide weak support for earlier reports that Mn excess increases aggression, primarily because rats fed high Mn were less active than those fed adequate Mn. No support was found for the hypothesis that Mn deprivation is associated with increased aggression. Findings expand knowledge of the functional role of dietary Mn, and its interaction with Ca, at physiological as well as toxicological concentrations. Predicted impact is redirection of future

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National Program(s): 107 100%

research on possible nutritional involvement in aggressive behavior to study the interaction of mineral nutrients.
Completed research showing that dietary selenium (Se) affects mood states of healthy adults. Men in the United States with typically adequate Se intakes and fed approximately 3 times the RDA for Se for 12 weeks reported less depression and mental confusion than men fed approximately one-third the RDA. Women in New Zealand with typically low Se intakes and supplemented daily with 40 ug Se reported more energy and confidence, less hostility, and a decrease in total mood disturbance after 15 weeks. Findings indicate a novel function for dietary Se that may be used to help establish Se requirements for adults. Findings were widely reported by the national media, followed by numerous inquiries from other researchers and the general public. Extension of this research to assess cognitive performance and brain electrical activity in relation to Se-induced mood changes is a major component of grant proposals recently submitted to the National Cattlemen's Beef Association, North Dakota Beef Commission, and USDA ARS National Research Initiative program.
Copper (Cu) and magnesium (Mg) are two minerals of potential relevance to behavior because of their importance in neurotransmitter metabolism and because previous studies have shown that dietary intakes of both minerals affect brain electrophysiology. Determined in mature rats that low dietary intake for 10 weeks of either Cu (0.05 versus 6.0 ug/g) and Mg (50 versus 500 ug/g) was associated with an increase in generalized activity. Low Cu intake also resulted in more stereotypic behavior during presentation of an auditory stressor, whereas low Mg intake resulted in increased stereotypic behavior regardless of the presence of the stressor. Low Cu intake was associated with poorer performance on measures of learning, whereas low Mg intake was associated with poorer performance on measures of memory. Neither Cu nor Mg showed strong effects on direct measures of anxiety; however, indirect measures of stressor effects during activity monitoring and memory testing suggest that both minerals may impact emotionality. Findings indicate that both Cu and Mg have functional consequences at the behavioral (and possibly emotional) level, which complement earlier findings of effects of these two minerals on brain physiology. Predicted impact is future research on neuropsychological function and behavior of humans.

What do you expect to accomplish during the next year?

During the next year, research will be completed on the interactive effects of zinc and copper on cognitive function of healthy postmenopausal women participating in a controlled metabolic unit study. Research will be completed on a collaborative study of the effects of low zinc intakes on cognitive processing efficiency and psychomotor skills of young men.
During the next year, research will continue on a long-term collaborative study investigating the effects of iron and zinc supplementation on the

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Accession: 0400106 Year: 98 Project Number: 5450-51000-019-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 90% 5.1.4.3 10%
National Program(s): 107 100%

neuropsychological function of young women living in Galveston, TX and Dunnedin, New Zealand.
Also completed during the next year will be a collaborative study of the relationship between cognitive function and vitamin B-12 status of Guatemalen school children.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

To meet the need for valid yet inexpensive and easy-to-use procedures to routinely assess the relationship between nutrition and behavior, a computer software package and associated procedures were developed to automate the administration of standardized neuropsychological tasks designed to assess a variety of cognitive processes (e.g., perception, attention, learning, memory and reasoning) and psychomotor and spatial skills. Initially designed for English speaking adults, tasks and instructions have recently been redesigned to be more image-based for use with children and non-English speaking persons. This technology and related methods represent significant contributions to research on the neuropsychological and behavioral effects of nutritional deficiencies and supplementation. Users of this technology are researchers in private industry and in state and federal governments, domestic and foreign, including the Department of Preventative Medicine and Community Health at the University of Texas Medical Branch at Galveston, Department of Internal Medicine at the Wayne State University School of Medicine, John Stuart Research Laboratories of the Quaker Oats Company, USDA, ARS Western Human Nutrition Research Center, Department of International Nutrition at the University of California at Davis, Chinese Academy of Preventative Medicine, PRC, and University of Otago, New Zealand.
Currently in progress are 6 collaborative research projects using this technology, supported by 5 granting agencies, and involving 6 researchers in 4 countries.
Continued updates and enhancements of this technology will ensure its durability. Lack of familiarity with behavioral and computerized testing, the need for careful training of test administrators, and the lack of age- and country-specific norms are current constraints on adoption of this technology.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Researchers Uncover Zinc Secrets for Children. Grand Forks Herald p. C1. October 7, 1998.
During the past year, more than 25 stories were presented in the popular media, including Agriculture Contact; Agricultural Research; American

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Health; Associated Press; Cooking Light; Council for Responsible Nutrition News; Energy Times; Fargo Forum; Fitness; Grand Forks Herald; Health; KDLR Radio; Los Angeles Times; Natural Health; Natural Remedies; Nutrition News; Parenting; PBS TV (Healthweek); Reader's Digest; Rodale Press; and Shape.

Publications:

01. PENLAND, J.G., SANDSTEAD, H.H., ALCOCK, N.W., DAYAL, H.H., CHEN, X.C, et al. 1998. Zinc and micronutrients affect cognitive and psychomotor function of rural Chinese children. FASEB J. 12:A649. (Abstract)

02. SANDSTEAD, H.H, PENLAND, J.G., ALCOCK, N.W., et al. 1998. Effects of repletion with zinc and other micronutrients on neuropsychologic performance and growth of Chinese children. Am.J.Clin.Nutr. 68:470S-475S.

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401450 Year: 98 Project Number: 5450-51000-023-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 80% 5.1.3.4 20%
National Program(s): 107 100%

Title: BIOCHEMICAL CONSEQUENCES OF SUBOPTIMAL DIETARY INTAKE OF TRACE ELEMENTS

Period Covered From: 04/97 To: 04/02

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

Zinc and copper are cofactors for a large number of enzymes that catalyze important biochemical reactions. The effects of low zinc and copper intakes on the in vitro activities of these enzymes is one paradigm that has been used for estimating human and animal requirements for zinc and copper. However, this paradigm has not provided sufficient information to define either explicit health effects of low zinc and copper intakes or the dietary requirements of these elements for health and optimal performance. A major reason for this insufficiency is the lack of knowledge regarding how reductions in the activities of zinc- and copper-dependent enzymes perturb cellular and organ function and the lack of knowledge regarding non-enzymatic roles of zinc and copper.
Animal models of copper and zinc deficiencies and cells cultured in media containing various concentrations of zinc and copper are used to investigate the various biochemical mechanisms underlying the functional consequences of low zinc and copper intakes and low cellular content of these trace elements. The influences of copper and zinc deprivation on the synthesis of bioactive molecules, transport mechanisms, transmembrane signaling, and mitochondrial function are described. Knowledge based on the descriptions of biochemical mechanisms for the functional outcomes of zinc and copper deficiencies can more precisely define the dietary requirements for zinc and copper for health and optimal performance during all stages of life in men and women.
Certain dietary nutrients such as copper and zinc interact in the gut to inhibit the absorption of each other. However, zinc affects copper absorption and utilization more than copper affects zinc. If a person consumes two to three times the requirement (RDA) of zinc, either from food or, more likely, from food supplements for an extended period, copper absorption could be reduced to such an extent that the person may develop a low grade copper deficiency. There are numerous brands of supplements sold in the market place that contain large amounts of zinc. At present, these zinc supplements and their contents are not regulated. Therefore, the basis for this problem is the need to understand the physiological effects of high zinc intakes on copper absorption and utilization. We are using a human intestinal cell model to determine the basic mechanisms involved in the copper-zinc absorption interaction. This information can be used to help set standards for zinc contents of food and food supplements, and for use in making recommendations for copper intake when dietary zinc might be in excess.

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National Program(s): 107 100%

How serious is the problem? Why does it matter?

Current information indicates that copper and zinc intakes for a large portion of the population are below the currently recommended amounts. In particular, lower than recommended copper intakes were found in every age-sex group surveyed in the United States. It is not known if low intakes of zinc and copper can have long-term health effects, particularly in the development of degenerative diseases of the cardiovascular and nervous systems and in fetal development. Knowledge regarding the biochemical mechanisms leading to negative health effects of low zinc and copper intakes can provide a basis for recommending dietary requirements that can slow or ameliorate the development of degenerative diseases and lower the burden of health care costs.
More than 80% of the diets consumed in the United States do not contain the recommended allowance for copper. Therefore, an intake of a small excess of zinc could easily promote or induce a mild to moderate copper deficiency. Copper is an essential nutrient for myriad physiological and biochemical processes, many of which are involved with development and function of the nervous system in the fetus. Although zinc is also required for development, it is extremely important to know the precise balance between the two nutrients so that an excess of one can be counterbalanced by the proper intake of the other.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107; Human Nutrition Requirements, Food Composition, and Intake (100%) Program Components; Changes in needs for nutrients throughout the life cycle, Definition of marginal or borderline deficiencies.
This research will develop information about the effects of zinc and copper deficiencies on biochemical functions that will facilitate the detection of marginal copper and zinc deficiencies and define the dietary requirements of these trace metals for health, development and optimal performance throughout the life cycle. The research will also provide information that can be used to assess the risk of chronic disease from subclinical zinc and copper deficiencies.

What was your most significant accomplishment this past year?

Copper deficiency increases the activities of manganese superoxide dismutase in liver and heart mitochondria and glutathione peroxidase in heart mitochondria. This finding demonstrates that mitochondria experience increased oxidative stress during copper deficiency. Furthermore, the increase in oxidative stress was accompanied by increased oxidative modifications of mitochondrial proteins. This indicates that

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National Program(s): 107 100%

even though manganese superoxide dismutase and glutathione peroxidase activities increased during copper deficiency, these compensatory increases in antioxidant protection were not sufficient to protect mitochondria from damage by oxygen radicals. Degeneration of mitochondrial function because of increased oxidative damage may partially explain the increased risk of chronic disease associated with copper deficiency. (W.T. Johnson)
Physiological concentrations of zinc, i.e., concentrations similar to those in the gut after a meal, can significantly affect the transport of copper across an intestinal cell monolayer. The model system used was a modified intestinal cell that is immortal, but functions the same as an intestinal cell in the body. Heretofore, it was thought that only very high concentrations of zinc would have a major effect on copper transport. (P.G. Reeves)

Describe your major accomplishments over the life of the project, including their predicted or actual impact

The effect of marginal copper deficiency during pregnancy on the expression of the alpha, beta, and gamma isoforms of protein kinase C in neonatal rat brain was examined. Copper deficiency reduced the rate of expression of the protein kinase C isoforms during the three weeks following birth and led to significant reductions of protein kinase C beta in the hypothalamus and protein kinase C gamma in the hypothalamus and cerebellum. Impact: Protein kinase C expression is a determinant of brain development. Impairment in the expression of protein kinase C isoforms may eventually explain how neurological function and intellectual development are affected in the offspring of mothers who are subclinically copper deficient during pregnancy and the perinatal period. (W.T. Johnson)
High concentrations of zinc in the diet, equal to two to three times the RDA that can be found in over-the-counter mineral supplements, can lower the copper status of humans and may eventually lead to signs of copper deficiency. It is hypothesized that this effect is caused by zinc altering the transport of copper through the intestinal epithelial cell. To study this phenomenon, a cell culture system was devised that uses an intestinal cell mimic derived from a human colon carcinoma cell, that when differentiated, has the same characteristics as an intestinal epithelial cell in the body. As predicted, moderate to high concentrations of zinc in the growth media of the cells inhibited copper transport. Impact: This work strongly suggests that zinc affects the activity of the two newly discovered copper transport proteins, Menkes and hCTR1, and, for the first time, suggests a plausible mechanism for the cause of low copper status in humans and animals fed excess zinc in their diets. (P.G. Reeves)
Another long-held theory concerning the effect of high concentrations of dietary zinc on the copper status of animals and humans, is that zinc-induced intestinal metallothionein (MT), a metal-binding protein, binds copper and prevents its absorption. This theory, which had begun to

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appear in text books, was challenged when we repeatedly showed in animal studies that there was no positive correlation between zinc-induced MT and the amount of copper in the intestine. We also found that MT induction during long-term feeding of high zinc diets was reduced, but the animal remained copper-deficient, which strongly suggested that MT induction in the intestine by zinc was not the cause of low copper status. More recently and most importantly, we showed that mice without the MT gene, and without the ability to produce MT, still become copper deficient when fed high zinc diets, thus providing further evidence that intestinal MT is not required for this process. Impact: This research challenged the current theory surrounding zinc-copper interaction at the intestinal level and demonstrated that MT was not a necessary component of this process. These findings have raised new questions as to the real function of intestinal MT in the regulation of mineral absorption and status and has opened up new avenues for the study of trace element absorption mechanisms and metabolism. (P.G. Reeves)

What do you expect to accomplish during the next year?

The effects of copper deficiency on the production of reactive oxygen species by liver and heart mitochondria will be examined. This study will test the hypothesis that low copper status is a determinant of oxygen radical production by mitochondria and that mitochondria are the main source of reactive oxygen species that cause intracellular damage during copper deficiency. (W.T. Johnson)
Copper deficiency may lower the expression of protein kinase C. Decreased expression of some isoforms of protein kinase C has been observed in primary human colon tumors and chemically-induced colon tumors in experimental animals. It will be determined if copper deficiency potentiates chemically induced carcinogenesis in rat colon by repressing protein kinase C expression. This will identify a potential role for dietary copper in reducing the risk for colon cancer. (W.T. Johnson)
Cellular copper status may affect the second messenger roles of oxygen radicals by enhancing their production. It will be determined if mitochondrial manganese superoxide dismutase is induced as a response to increased oxygen radical production during copper deprivation in cultured HL60 cells. The results of this investigation will provide a foundation for investigating the relationship between copper status, oxygen radicals, and the gene regulation of proteins that respond to oxidative stress. (W.T. Johnson)
Studies will be designed to determine if zinc is affecting the physiological function of the cell membrane transporters for copper. Two newly discovered transporter proteins, Menkes and hCTR1, are essential in getting copper into and across the cell. The question to be answered, Does excess zinc inhibit the transporter activities of these proteins, or is it related to the production of the proteins in the first place? Answers to these questions would significantly advance the knowledge about

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the regulation of transport of copper and about trace elements in general. (P.G. Reeves)

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

Results from studies are published in peer-reviewed scientific journals. Upon publication, the methodology, data, and interpretations of the data are immediately available to scientists and other interested parties. In addition, experimental results and educational information pertinent to public interests are made available through the local newspaper, The Grand Forks Herald, and statewide through the Interactive Video Network, North Dakota State University Extension Service Continuing Education Program.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Popular publication of this work has appeared in the local newspaper, The Grand Forks Herald, under the title "Think Zinc!" and "Copper: A Nutrient With a History". Presentations of part of this work were also given this year at the annual meetings of the Federation of American Societies and Experimental Biology (FASEB) in San Francisco, CA, and at FASEB Summer Conference in Wilsonville, OR.

Publications:

01. REEVES, P.G., BRISKE-ANDERSON, M. and JOHNSON, L. 1998. Physiologic concentrations of zinc affect the kinetics of copper uptake and transport in the human intestinal cell model, Caco-2. J.Nutr. 128:1794-1801.

02. REEVES, P.G. 1998. Copper metabolism in metallothionein-null mice fed a high-zinc diet. J.Nutr.Biochem. 9:598-601.

03. JOHNSON, W.T. and LOZANO, A.A. 1998. Maternal copper deficiency alters the distribution of protein kinase C isoforms in neonatal rat brain. FASEB J. 12:A200.

04. REEVES, P.G. and BRISKE-ANDERSON, M. 1998. Physiologic concentrations of zinc affect the rate and kinetics of copper uptake, transport and release in Caco-2 cells. FASEB J. 12:A242.

05. BRISKE-ANDERSON, M., NEWMAN, S.M. and REEVES, P.G. 1998. Support membrane porosity influences the morphological uniformity of Caco-2 cells in culture. FASEB J. 12:A370.

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National Program(s): 107 100%

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

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Accession: 0147990 Year: 98 Project Number: 5450-51000-023-03 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.2 70% 5.2.2.2 30%
National Program(s): 107 100%

Title: HEALTH EFFECTS AND BIOAVAILABILITY OF CADMIUM FROM SUNFLOWER SEED KERNELS:A HUMAN STUDY

Period Covered From: 01/98 To: 10/98

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

The major problem centers around the fact that some crops, sunflowers in particular, that are grown in the upper Mid-Western United States contain a higher amount of cadmium than similar crops grown in other parts of the country. Cadmium is a mineral that is toxic at relatively low intakes. Two major questions need to be answered: 1) Is the cadmium in sunflower kernels available for absorption into the body, and 2)if so, does it affect the body burden of cadmium and the health of the consumer?
We are trying to resolve the first question by actually assessing the absorption of cadmium into the body by feeding kernels containing a stable isotope of cadmium and determining the amount of unabsorbed labeled Cd coming out in the feces. The difference between intake and output in the feces is a measure of absorption. The second question is being assessed by feeding sunflower kernels containing cadmium for a long period (48) weeks and determining if parameters that indicate body accumulation of the cadmium are affected. These include cadmium concentrations in urine and red cells. Increased concentrations indicate that cadmium may be accumulating in the body and increasing the body burden.
An additional problem involves trade restrictions on food commodities containing cadmium. At present, the European markets import sunflower kernels only if their cadmium concentration is no higher than 0.6 mg/kg. These countries propose to lower this concentration considerably. If they do, this action will severely reduce the sale of U.S. sunflower kernels to these markets. Is this proposed restriction warranted? This can be resolved by having knowledge of the availability of cadmium from the sunflower kernels, and knowledge about the effects of long term consumption of the kernels, so that rational decisions can be made on the allowable amounts of cadmium in food commodities.

How serious is the problem? Why does it matter?

The seriousness of this problem is two-fold. Because cadmium is best known as a toxin, the question that is ever present: Does the intake of sunflower kernels containing a small amount of cadmium increase the body burden of cadmium, and does this, in turn, affect the health of the individual? Secondly, because of trade restrictions placed on cadmium content of food commodities, such as sunflower kernels, by European

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National Program(s): 107 100%

Markets, is the amount and availability of cadmium in this commodity sufficient to warrant further restrictions, and a possible loss of trade by the U.S. Agricultural Community?

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition, & Food Safety.
This research will allow us to establish safe and adequate intakes of cadmium for optimal health and bodily functions.

What was your most significant accomplishment this past year?

A two year long human feeding study was finished. The study tested the null hypothesis that long-term feeding (48 wk) of sunflower kernels to humans would not affect their cadmium status as determined by current non-invasive and relevant biological tests. Preliminary data show that cadmium status indicators were not affected by feeding the cadmium-containing sunflower kernels.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

Accomplishment: Over the life of the project, animal studies have shown that cadmium from a diet containing 20% sunflower kernels is less available than from one containing no kernels. Studies with human volunteers have shown that individuals who report a regular consumption of more than 1 ounce of kernels/wk are more likely to have higher cadmium intakes than those who report eating less than one ounce/week. However, no adverse health affects were found as a result of consuming these or higher amounts of sunflower kernels. Two human studies to assess the availability of cadmium from sunflower kernels on a long-term basis, and to test the effects of long-term controlled feeding of kernels, have shown that the intestinal absorption of cadmium from the kernels ranges from 2 to 10%, but that no increase in body burden or adverse health effects was found when subjects consume up to 9 ounces of kernels/wk for 48 weeks.
Impact: The CODEX Alimentarius Commission of the United Nations Committee on Food Additives and Contaminants will meet in 1999 to discuss setting limits on the amount of cadmium to be allowed in imported sunflower kernels. The information gathered from our studies will be used in those discussions, and will provide a more rational approach to decision making about the allowable amounts of cadmium in sunflower kernels as well as other food commodities. The International Lead/Zinc Research Organization, Inc., and The National Sunflower Association supported part of this work (approximately $32,000 and $5,000, respectively). The National Program Staff provided additional support of $140,000.

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What do you expect to accomplish during the next year?

At the present time, there are no direct funds to continue support of this project. Hence, it is doubtful that any new research proposals will be forthcoming in the next year. Most efforts will be centered on wrapping up the last human study and preparing manuscripts for publication and presentation.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

The findings brought forth in this research have been presented to the Food and Drug Administration, the agricultural industry, other scientists, and have been published in scientific journals. All information is immediately available for adoption. In addition, some of the findings have been disseminated in the form of presentations at national and international scientific meetings.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

As a result of this research, the incumbent was invited to review the progress of the research at numerous meetings of the National Sunflower Association. In addition, this work was presented to the Grand Forks Human Nutrition Research Center's Focus Group, which was made up of members from many disciplines, including commodity groups, local business groups, city and university governancies, and representatives from the federal government.

Publications:

01. REEVES, P.G. and VANDERPOOL, R.A. 1998. Organ content and fecal excretion of cadmium in male and female rats consuming variable amounts of naturally occurring cadmium in confectionery sunflower... J.Nutr.Biochem. 9:636-644.

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149978 Year: 98 Project Number: 5450-51520-011-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.2.2.2 25%
National Program(s): 107 100%

Title: BIOCHEMICAL, PHYSIOLOGICAL, AND NUTRITIONAL ROLES OF CERTAIN ULTRATRACE ELEMENTS

Period Covered From: 03/96 To: 03/01

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

Numerous mineral elements consumed in ultratrace amounts have been considered to be of possible importance in the prevention of disease with nutritional roots, or for the enhancement of health and longevity. Many of these elements, because of some promising physiological or clinical finding, most often in an animal model or a special human situation, are often ostentatiously promoted for the purpose of financial gain. Health and nutrition professionals often have these elements brought to their attention by clients because these promotions often claim that supplements of these mineral elements can prevent some feared diseases such as cancer, heart disease and loss of cognitive function, or can enhance physical appearance. Among these elements are those for which there is circumstantial evidence for essentiality, but are not unequivocally accepted as essential because they lack a defined specific biochemical function in higher animals. Some of these elements are inappropriately promoted (e.g., vanadium) such that intakes detrimental to health may be occurring. Other ultratrace elements apparently have health benefits that are now only being discovered or defined (e.g., boron) and thus increased recognition. Therefore, credible and data-supported dietary recommendations need to be developed for some ultratrace elements so that they can be used by people to assure health and well-being. Additionally, regulatory agencies will increasingly consider nutritional and health benefits of nutrients, especially minerals, so that risk assessments and toxicological standards do not conflict with amounts beneficial to health, in addition to not causing economic burdens to reduce environmental exposure to amounts that may do more harm than help in preserving health and well-being.
Animal and human experiments are and will be conducted to define the biochemical and physiological roles of various ultratrace elements, including arsenic, boron, nickel, silicon, and vanadium. The basic approach will be to feed experimental animals or human volunteers diets that contain amounts of specific ultratrace elements and other selected nutrients and non-nutrients (postulated to affect the metabolism and utilization of specific ultratrace elements) which are accurately controlled and systematically varied. The response of the animals and humans to the dietary manipulations will be ascertained by evaluating various appropriate biochemical, physiological and anatomical variables. Biochemical and molecular biological methods will be used to define exact biochemical roles of specific ultratrace elements. The findings will

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National Program(s): 107 100%

help in the determination of the nutritional importance of specific ultratrace elements and whether their dietary intake influences the occurrence of chronic disease.

How serious is the problem? Why does it matter?

A paradigm shift has occurred in the setting Recommended Dietary Allowances which have been renamed Dietary Reference Intakes (DRIs). The dominant role of deficiency in the determination dietary guidelines is now complemented by the total health effects of dietary components or nutrients. Thus, where sufficient data for efficacy and safety exists, the setting of new DRIs will consider reduction in the risk of chronic disease in addition to preventing deficiency pathology. This is important because dietary factors are associated with 5 of the 10 leading causes of death, including coronary heart disease, certain types of cancer, stroke and atherosclerosis. Among those diseases that are linked strongly to diet, the cost of treatment and care in the United States exceeds $200 billion per year. Recognition that nutrition is important in health promotion and disease prevention, has spawned a plethora of health enhancing foods and supplements, now often called "functional foods" or "nutraceuticals" that represent an exploding market in the United States conservatively estimated at $29 billion a year. Many of the health claims for these health enhancing foods and supplements, however, have not been substantiated by basic research and human feeding trials. Many of the health claims include the use of ultratrace elements because their reduced intakes have been associated with one or more chronic diseases. Thus, research is needed to establish which foods and in what amounts will provide clinically important ultratrace elements in amounts that promote health and disease prevention, and to determine safe intakes of specific ultratrace elements so that the setting of reasonable toxicological standards can be accomplished. This information should result in policies and programs that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care and environmental exposure protection expenditures.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research relates to the National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The research is related to the specific National Program Components of 1) Changes in needs for nutrients throughout the life cycle. 2) Function and metabolism of nutrients for cognitive and physical development. 3) Definition of marginal deficiencies or borderline deficiencies. 4) Nutritional needs of a diverse population. The research will develop information about the effects of deficiency or imbalance of specific nutrients on biochemical, structural, physiological and psychological functions to facilitate their

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National Program(s): 107 100%

detection and prevention, and to define requirements for health and well-being. It also will establish safe and optimal intakes, and the roles in risk or prevention of diet-related disorders, of ultratrace elements.

What was your most significant accomplishment this past year?

Dietary boron, fed in nutritional amounts, was found to increase circulating concentrations of natural killer cells and cytoxic/suppressor T lymphocytes, two critical components of the immune system. The effect of boron on these immune cells was beneficial because it resulted in the reduction of body tissue swelling after experimental induction of an inflammatory condition. This finding indicates that nutritional or physiological amounts of boron modulate the response of key immune cells to antigens and help control the normal inflammatory response. Certain intakes of boron apparently are needed for optimal immune function and appropriate inflammatory response. (Curtiss Hunt)
Arsenic deprivation depresses the activity of the enzyme betaine homocysteine methyltransferase in rats regardless of methyl status which generally markedly affects how animals respond to low dietary arsenic. The finding indicates that arsenic is having a direct, not an indirect, effect on the enzyme and provides support for the nutritional essentiality of arsenic. (Eric Uthus)
Vanadium status affected the response of rats to N-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide formation. When compared to vanadium deprived animals, the response of rats to nutritional amounts of vanadium was different than when supra nutritional amounts of vanadium were fed. This was most apparent in thyroid hormone status, energy metabolism, and oxidative metabolism status indicators. The findings suggest that vanadium has a biological role in addition to pharmacological activity, involving a reactive oxygen species and supports the concept that vanadium is an essential nutrient, but is needed in very low amounts. (Forrest Nielsen)

Describe your major accomplishments over the life of the project, including their predicted or actual impact

Normal or nutritional dietary intakes of boron help control the normal inflammatory process and therefore reduce the development of inflammatory disease. The impact of this accomplishment is that it may lead to the development of a new dietary regimen for intakes of boron that could reduce the significant prevalence of inflammatory disease (i.e., rheumatoid arthritis) in the American population. (Curtiss Hunt)
Vanadium was found to be a nutritionally important element involved in thyroid hormone and glucose function or metabolism. However, the findings indicated that the amount of vanadium needed to assure appropriate thyroid hormone and glucose metabolism was extremely small and that supra nutritional amounts, similar to amounts provided by supplements available

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National Program(s): 107 100%

to people, induced changes that could be construed as not beneficial. This suggests that some over-the-counter supplements sold as anabolic or anti-diabetic agents could be detrimental to health. (Forrest Nielsen)
Arsenic was demonstrated to have a physiological role that affects methionine metabolism, most likely, methionine recycling. Arsenic deprivation increases liver S-adenosylhomocysteine (SAH), decreases S-adenosylmethionine (SAM), and thus, decreases the ratio of SAM to SAH. A low SAM/SAH ratio has been associated with an increased risk of certain types of cancer. These findings in addition to others obtained during the life of the project indicate that arsenic is a nutritionally essential ultratrace element whose need is influenced by nutritional stressors that affect sulfur amino acid metabolism. This accomplishment has been used to challenge the need to establish a extremely low toxicity threshold for arsenic in drinking water (i.e., decreasing the allowable concentration from 50 to as low as 5 ug/L) and food and thus save a significant (billions of dollars) cost to the public. (Eric Uthus)
Fuzzy logic was first used in the derivation of nutrient requirements. Other novel mathematical approaches for determining nutrient requirements have also been proposed. An initial nonlinear model was developed to account for interactive effects of other nutrients and non-nutrients on requirements. These approaches are more objective and can easily be adapted as new information becomes available for the derivation of recommended intakes. Also, because they account for interactive effects (synergistic or antagonistic), the models can be tailored to a specific group of individuals. (Eric Uthus)
Nickel and interactions among nickel, folic acid and vitamin B-12 were found to affect the vitamin B-12 and folate dependent pathway of methionine synthesis from homocysteine. The findings indicate that nickel has an essential biological role which could influence the risk factors associated with coronary heart disease of decreased circulating vitamin B-12 and folic acid, and increased circulating homocysteine. Moreover, because nickel deprivation exacerbates folic acid deficiency, nickel nutriture might be a factor in some cases of neural tube defects caused by folic acid deficiency. (Eric Uthus and Forrest Nielsen)

What do you expect to accomplish during the next year?

Further immune challenge studies will be conducted to determine the mechanism for the marked response of specific types of immune function cells to boron deprivation; findings should help determine the boron requirement for normal immune function and establish functional indicators of boron status. (Curtiss Hunt)
Animal experiments will be done to ascertain whether homocysteine status affects the response to nickel deprivations, or conversely, whether nickel has a function that affects homocysteine metabolism and thus is factor in the association between elevated circulating homocysteine and the risk of heart disease. The experiments will also help determine whether nickel

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has a function at the cell membrane level affecting signal transduction involving ionized calcium and/or phosphate moieties. (Forrest Nielsen)
Animals fed amino acid diets will be used to show that arsenic directly affect the cycling of methionine. This will help direct further efforts in the determination of the exact site at which arsenic has an essential or beneficial biological effect. (Eric Uthus)
By using the isotope nickel-63, find, isolate and characterize specific nickel containing proteins. Also, through the use of this isotope, complete the development of a mathematical model of nickel metabolism in rats. Findings should help establish the nutritional essentiality and importance of nickel (Eric Uthus and Forrest Nielsen)
Derive a mathematical approach that uses easily accessible blood variables to predict mineral status of animals and humans. This approach will be useful for determining mineral requirements of specific groups or individuals. (Eric Uthus)

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

Updated a special boron-low animal diet developed under the auspices of the CWU predecessor of this CWU, and provided the diet formulation to others; this includes US Borax, Valencia, CA, to assist in their efforts to demonstrate a boron requirement for mammalian reproduction, and the Stover Group, Stillwater, OK, to assist in their efforts to demonstrate a boron requirement for amphibian reproduction.
Information about the nutritional or beneficial aspects of ultratrace elements as it becomes available is routinely transferred to a variety of customers. The customers include risk assessments groups such as the Environmental Protection Agency, National Institutes of Environmental Health Sciences, and World Health Organization's International Programme on Chemical Safety through direct contact or organized workshops; the public through web pages of professional organizations and via the popular media, and other scientists through presentations at national and international meetings and professional publications.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

1. Presentation of the invited plenary talk "The Importance of Making Dietary Recommendations for Elements Designated as Nutritionally Beneficial, Pharmacologically Beneficial, or Conditionally Essential" at the Fifth International Conference of the International Society of Trace Element Research in Humans, Lyon, France (Forrest H. Nielsen).
2. "It Takes More Than Calcium to Keep Bones Healthy," article published by the Grand Forks Herald newspaper and on the Grand Forks Human Nutrition

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Research Center's Home Page. (Curtiss Hunt)
3. "The Big Picture: Groundbreaking News on Boron's Nutritional Essentiality," Borax Pioneer, No. 12, 1998. (Pioneering work of Forrest Nielsen and Curtiss Hunt featured.)

Publications:

01. NIELSEN, F.H. 1998. Ultratrace elements in nutrition: Current knowledge and speculation. J. Trace Elem. Exp. Med. 11:251-274.

02. NIELSEN, F.H. 1998. Ultratrace minerals, pp. 283-303. IN: M.E. Shils, J.A. Olson, M. Shike & A.C. Ross (eds.), Modern Nutrition in Health and Disease, 9th Ed., Williams & Wilkins, Baltimore.

03. NIELSEN, F.H. 1998. Ultratrace Elements, Physiology, pp. 1884-1897. IN: M. Sadler, J.J. Strain, B. Caballero (eds.), Encyclopedia of Human Nutrition, Academic Press, London.

04. HUNT, C.D. 1998. Copper and boron as examples of dietary trace elements important in bone development and disease. Cur. Opinion Orthop. 9:V:28-36.

05. NIELSEN, F.H. and UTHUS, E.O. 1998. Dietary vanadium affects carbohydrate and thyroid metabolism in the BB rat. Proc. North Dakota Acad. Sci. 52:43.

06. ZASLAVSKY, B. and UTHUS, E.O. 1998. Interrelation between zinc and iron nutriture in rats. Proc. North Dakota Acad. Sci. 52:44.

07. NIELSEN, F.H. 1998. Manganese deprivation is not a propitious stressor of boron metabolism or nutrition. FASEB J. 12:A205.

08. HUNT, C.D. 1998. Dietary boron enhances the effects of exercise training on bone calcium, phosphorus, and magnesium concentrations in the rat. FASEB J. 12:A205.

09. HUNT, C.D. 1998. Dietary boron as a regulator of enzymatic activity in higher animals and humans. J. Trace Elem. Exp. Med. 11:388.

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Publications: (Continued)

10. UTHUS, E.O. and POELLOT, R.A. 1998. High dietary sodium arsenite affects kidney trace element concentrations. FASEB J. 12:A221.

11. ZASLAVSKY, B., KLEVAY, L.M. and UTHUS, E.O. 1998. Nonlinear analysis of the interaction between copper and zinc in the rat. FASEB J. 12:A665.

12. MEACHAM, S.L. and HUNT, C.D. 1998. Copper content of common American foods. FASEB J. 12:A199.

13. LANOUE, L., STRONG, P.L., HUNT, C.D., and KEEN, C.L. 1998. Effects of boron deficiency and toxicity on preimplantation mouse embryos. Defining the limits of boron nutriture using an in vitro model. FASEB J. 12:A205.

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401535 Year: 98 Project Number: 5450-51520-011-03 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 50% 5.2.2.2 50%
National Program(s): 107 100%

Title: THE NUTRITIONAL ROLE OF BORON IN THE INHIBITION OF SERINE PROTEASES

Period Covered From: 04/98 To: 12/98

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

The normal inflammatory response serves to focus host defenses at a site of tissue injury or infection. Thus, under normal conditions, the defense mechanism of inflammation serves a vital function. Typically, elimination of antigens proceeds without evidence of clinically detectable inflammation. Excessive inflammation leads to inflammatory disease (for example, rheumatoid arthritis). This can occur when the immune system has encountered either an unusually large amount of antigen, antigen in an unusual location, or antigen that was difficult to destroy. As first shown in this laboratory, dietary boron reduces the onset and severity of induced arthritis in rats. Also, certain boron compounds are potent in vitro inhibitors of several enzymes that regulate the normal inflammatory reaction. Therefore, the focus of this project is to identify inflammatory mediators that interact with boron. This approach will help establish the specific function of boron in humans and subsequently be useful in identifying marginal or borderline boron deficiency.
Based on molecular structure, several classes of compounds predicted to interact with boron were selected and assessed to determine the exact role of boron in regulation of the inflammatory response. The basic approach will be to determine the in vitro binding affinities of these compounds to boron. Compounds with physiologically significant boron binding affinities will be investigated as potential indicators of boron status by in vivo measurement of these compounds after extraction from tissues of animals fed boron deficient diets.

How serious is the problem? Why does it matter?

A paradigm shift has occurred in the setting of the Recommended Dietary Allowances which have been renamed the Dietary Reference Intakes (DRIs). The new DRI values reflect the latest understanding about nutrient requirements based on optimizing health in individuals and groups, not just the prevention of nutrient deficiency. Therefore, establishing the new DRIs will involve consideration of the effect of the nutrient on reduction in the risk of chronic and other diseases and conditions. Inflammatory diseases cost Americans billions of dollars yearly in treatment and loss of productivity. Rheumatoid arthritis in particular is a painful, chronic recurrent, systemic inflammatory disease that affects

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1-3 percent of Americans. Thus, prevention or significant amelioration of inflammatory diseases including rheumatoid arthritis by relatively simple dietary means, would have significant impact. There is a high probability that normal amounts of dietary boron will significantly ameliorate symptoms of rheumatoid arthritis in humans based on its known effect on cartilage development and maintenance and influence on the progression of experimental rheumatoid arthritis in animal model systems. Identification of biomolecules that interact directly with boron will serve to define borderline boron deficiency and develop indicators of boron deficiency.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research relates to the National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The research is related to the specific National Program Component of definition of marginal deficiencies or borderline deficiencies. The research is especially suited to defining borderline boron deficiency and to develop indicators of boron deficiency.

What was your most significant accomplishment this past year?

An in vitro model system was developed and implemented to demonstrate the direct binding of boron to biomolecules of considerable physiologic importance. This included the investigation of a signal hormone, called diadenosine tetra-phosphate (A**2P**4), that regulates cell proliferation and stress response. Compounds like A**2P**4 have two adjacent sets of hydrogen-oxygen that point in the same direction (called cis-diol compounds) and therefore can bond with boron. Boron binding to this compound was determined by measuring its migration time through an electrically charged solution. An increase in migration time through the solution indicates that the molecule binds boron. As predicted, the migration time of A**2P**4 increased as concentrations of boron were increased. The increase in migration time indicates that boron binds to this important molecule that controls cell growth and the response of the cell to stress.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

This is a new project started in April 1998.

What do you expect to accomplish during the next year?

A series of in vitro experiments will be conducted to further characterize the interaction between boron and ribonucleoside phosphates with special emphasis on diadenosine tetra-phosphate that is known to act as a signal

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National Program(s): 107 100%

hormone to regulate cell proliferation and stress responses. Based on the outcome of these experiments, the first attempts will be made to determine inflammatory cell concentrations of relevant ribonucleosides in boron-deprived experimental animal models.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

None.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

None.

Publications:
01. None.

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400356 Year: 98 Project Number: 5450-51530-003-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

Title: MINERAL ELEMENTS, PHYSIOLOGICAL FUNCTION & PERFORMANCE AND BODY COMPOSITION

Period Covered From: 03/96 To: 03/01

Would you like to terminate this Project? N

Progress and Outcomes:
What major problem or issue is being resolved and how are you resolving it?

Recommendations for the dietary intake of mineral elements, with an emphasis on zinc and copper, based on the promotion of health and optimal biological function are generally lacking. Studies designed to examine the effects of restricted dietary intakes of mineral elements on physiological function are needed to ascertain appropriate amounts of mineral elements in the diet to maintain health and to facilitate the attainment of genetic potential of biological functions. One factor in delineating appropriate dietary mineral intakes is assessment of food-borne factors that affect the absorption and utilization of dietary minerals.
Studies are conducted in animals and humans. Graded intakes of dietary zinc and copper are fed and physiological functions are monitored to delineate intake amounts that affect physiological function with an emphasis on energy utilization, work performance and heat production. Other studies are undertaken in which animals are fed diet low in iron, then given diets containing varied amounts of iron (low and adequate) and different types (saturated and polyunsaturated) of dietary fat. Changes in iron status and hematology, as well as changes in bone mineral content, are determined.

How serious is the problem? Why does it matter?

There is considerable debate regarding the amount of dietary copper and zinc for health maintenance and optimal biological function. Previous approaches focused on relatively insensitive measures of nutritional adequacy (chemical balance). By relating dietary mineral intakes to measurements of biological function, such as energy utilization, work production and heat generation, suggestions for dietary mineral intakes are made in reference to quality and quantity of life. Much of this research is requested by physically active individuals who seek to optimize physiological function without the use of dietary supplements and health managers who seek to minimize health care costs of the American public.
Iron deficiency is the single most prevalent nutritional deficiency in the world. Attempts to fortify food products have been unsuccessful in ameliorating this pervasive nutritional problem among females. In

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parallel with the incidence of iron deficiency, dietary fat consumption patterns indicate an increase of polyunsaturated fat intake. Studies indicate that the type of dietary fat affects iron, specifically non-heme iron, absorption and utilization. Polyunsaturated fat reduces and saturated fat, specifically stearic acid, promotes iron utilization. Because stearic acid is neutral to serum cholesterol and lipoprotein cholesterol concentrations, it offers practical benefits in ameliorating iron deficiency in animals and humans.

How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The specific Program Components include Definition of Marginal or Borderline Deficiencies- Biochemical and Health Consequences of Suboptimal Trace Element Intake, Methods of Nutritional Status Assessment, and Function and Metabolism of Nutrients for Cognitive and Physical Development.
This research will acquire information about the effects of graded trace element deficiencies, emphasizing zinc, copper, magnesium, iron, and chromium, on biochemical measurements and physiological functions. This information will facilitate the detection of marginal mineral deficiencies and define dietary requirements of these minerals elements for the development and maintenance of health and optimal function throughout the life cycle. The research will provide needed information that can be used to assess the risk of chronic diseases and impairments in subtle physiological functions that arise from mild and moderate mineral element deficiencies.

What was your most significant accomplishment this past year?

Restricted dietary zinc adversely affected energy utilization during exercise in young men. As compared to a dietary zinc intake of 18 mg/d, 3 mg zinc daily was associated with significant alterations in energy production and respiratory function during progressive peak exercise on a cycle ergometer. The mechanism of this impairment was a significant decrease in red blood cell carbonic anhydrase activity with restricted dietary zinc. Carbonic anhydrase is a zinc-containing enzyme with the specific function of transporting carbon dioxide from cells to the lungs for excretion. Zinc deficiency was confirmed with a significant loss of zinc, negative zinc balance, and decreased serum zinc concentration when dietary zinc was restricted. These findings provide the first evidence of impaired physiological function when dietary zinc is fed in an amount similar to that consumed by some physically active individuals. Also, the finding of decreased carbonic anhydrase activity in the red blood cell in response to low dietary zinc suggests that the activity of this zinc-containing enzyme may be a new blood biochemical marker for assessment of

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National Program(s): 107 100%

human zinc nutritional status.

Describe your major accomplishments over the life of the project, including their predicted or actual impact

Restriction of dietary copper resulted in altered temperature regulatory function of rats acutely exposed to cold air. Copper deficiency was associated with an increase rate of loss of body temperature, decreased enzymatic conversion of the active form of thyroid hormone, triiodothyronine, from thyroxine, and decreased activity of the rate limiting enzyme dopamine beta hydroxylase, a copper-containing protein, needed for increased production of norepinephrine, the key regulator of heat production. A key finding was the identification of decreased transcription and translation of uncoupling protein that is required for heat production and thermogenesis in brown adipose tissue. The lack of induction of uncoupling protein formation is caused by a depressed expression of the genetic message for a specific heat shock protein, HSP 70, in copper deficiency. Importantly, the adverse effects of copper deprivation are ameliorated with copper repletion within three days of copper supplementation. Impact: These findings provide important information explaining the mechanism of copper in regulation of energy metabolism, and explain why humans with an inborn inability to absorb copper suffer from hypothermia despite adequate hemoglobin concentration.
Supplementation of men with chromium picolinate while participating in controlled resistance training failed to demonstrate increased strength gain, facilitate loss of body fat and enhance muscle mass accretion. Impact: The results of this controlled study served as the basis for the Federal Trade Commission to rule that claims of propitious effects of chromium picolinate supplementation were without scientific basis. Similarly, the U.S. Pharmacopeia has concluded that chromium picolinate does not promote weight loss, facilitate body fat loss or promote muscle mass gain.
Consumption of dietary magnesium in amounts generally consumed by U.S. women resulted in alterations in energy production during submaximal exercise. Postmenopausal women fed 150 mg of magnesium daily demonstrated an increased requirement and elevated heart rates during submaximal exercise on a cycle ergometer as compared to diet providing 350 mg magnesium daily, the recommended dietary intake. Magnesium deficiency was documented with increased losses of magnesium and altered blood ionized magnesium, that is consistent with increased mobilization of magnesium from bone, and decrease skeletal muscle magnesium. Impact: These findings provide the first evidence of diet-induced magnesium deficiency in otherwise healthy adults, and demonstrate that dietary magnesium intake consistent with amounts generally consumed by a majority of U.S. women are inadequate to support moderate intensity physical activities of daily life. Because significant physiological impairments were found at magnesium intakes consistent with national estimates of usual intake,

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there is a need for increased public health education to bolster with dietary magnesium intakes to maintain physiological function and health. Stearic acid promotes non-heme iron absorption and utilization in iron-deficient animals. Studies in rats and canines repeatedly showed that stearic acid enhanced the uptake and transfer of non-heme iron from the intestinal mucosa and increased red blood cell volume and hemoglobin concentration. This beneficial effect occurred at high (30%) and moderate (20%) intakes of stearic acid. Furthermore, the enhancement of iron metabolism with stearic acid did not adversely impact calcium or magnesium status. Impact: Stearic acid may be the uncharacterized component of meat, the "meat factor", that promotes non-heme iron utilization. Although saturated fatty acids generally increase atherogenic risk by increasing serum cholesterol and low density lipoprotein cholesterol concentrations, stearic acid has no adverse effects on cholesterol or lipoproteins. Use of stearic acid in recipes containing non-meat foods may reduce the incidence of iron deficiency anemia in humans.

What do you expect to accomplish during the next year?

Develop, validate and implement a method for the use of tetrapolar bioelectrical impedance analysis for the assessment of regional muscle mass in humans. A model will be developed in weight-stable adults for determination of upper arm and thigh muscle mass. This model will be tested in overweight women undergoing controlled weight loss by food restriction and exercise. If this approach is successful, a practical method will be available for generalized use in the routine assessment of human nutritional status.
Evaluate the effects of chromium picolinate supplementation on mineral status and body composition of women consuming controlled diets. This study will test the hypothesis that chromium picolinate adversely affects iron metabolism; it will also evaluate the independent effect of picolinate per se on mineral metabolism. This project is novel because it will be the first study to examine potential effects of chromium picolinate in subjects with controlled energy intake and expenditure.
Examine the hypothesis that the copper requirement to maintain bone mineral content is increased during weight loss. Previous trials have shown a strong tendency for supplemental copper to attenuate bone mineral loss during weight loss. The current study will provide needed data to test the hypothesis that copper is needed to maintain bone mass.
Determine the effect of tissue copper depletion independently of anemia on the metabolic perturbation in energy metabolism of copper-deficient rats.
Develop a new approach to normalize oxygen carrying capacity of copper-deficient rats and examine the effects of copper deficiency on resting energy metabolism and substrate utilization in rats.
Evaluate blood cell populations as potential markers of dietary chromium adequacy. Preliminary studies are planned in which rats will be fed diets containing graded dietary chromium, both amounts and chemical forms, on

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the chromium concentrations of different populations of white blood cells and platelets. If successful, a double-blind cross-over trial of chromium supplementation of human subjects will be planned and initiated.

What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry,farmer,other scientists)? What are constraints, if known, to the adoption & durabiility of the technology

Information about the basic and applied aspects of the tetrapolar bioelectrical impedance method have been transferred to the National Institutes of Health and the Center for Disease Control and Prevention in multicenter collaborative projects. These projects involve the development and validation of body composition prediction models that use impedance and the application of these models to data acquired during the National Health and Nutrition Examination Survey (NHANES III) to derive national body composition norms. Information about the use and validity of the impedance method has been shared with Serono Pharmaceuticals for use of impedance technology in clinical trials of new drugs and Braun Industries for development of commercial devices for human body composition assessment. Results from studies are published in peer-reviewed scientific journals. Upon publication, the methodology, data, and interpretations of the data are immediately available to scientists and other interested parties. In addition, experimental results and educational information pertinent to public interests are made available through the local newspaper, The Grand Forks Herald, and statewide through the Interactive Video Network of the North Dakota State University Service Continuing Education Program.

List your most important publications and presentations,and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Public presentation, "Vitamin and Mineral Metabolism and Exercise Performance," at Gatorade Sports Science Conference on The Metabolic Bases of Exercise and Sport Performance, June 25-29, 1998. Popular publication in local newspaper, Grand Forks Herald, "Working Off the Holidays", January 11, 1998. Numerous stories in lay press including Women's Day, Rodale Press, Beef Today, Women's Sport, Sports for Women, Mademoiselle, Tufts University Health & Nutrition Newsletter, Mead Johnson Pharmaceuticals, Bristol-Myers, Cooking Light.

Publications:

01. SIDERS, W.A., LUKASKI, H.C., BOLONCHUK, W.W. and LYKKEN, G.I. 1998. Association of dominant somatotype of men with body structure, function and nutritional status. Proc. N.D. Acad. Sci. 52:41. (Short communication)

02. LUKASKI, H.C. 1998. Requirements for clinical use of bioelectrical impedance analysis. Proceedings of the Tenth International Conference on Electrical Bioimpedance, pp. 17-22. (Abstract)

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National Program(s): 107 100%

Publications: (Continued)

03. CHUMLEA, W.C., GUO, S.S., HEYMSFIELD, S., LUKAKSI, H. et al. 1998. Bioelectrical impedance (BIA) prediction equations for nationally representative NHANES III BIA data. Int.J.Obesity 22:S211. (Abstract)

04. GUO, S.S., CHUMLEA, W.C., HEYMSFIELD, S., LUKASKI, H.C., et al. 1998. The US national distributions of body fatness. Int.J.Obesity 22:S190. (Abstract)

05. CLARKSON, P., LUKASKI, H.C., and WILLIAMS, M.H. 1997. Chromium Supplements: Inconclusive evidence on the effect of chromium.... American College of Sports Medicine. November. Indianapolis, IN. (Policy Statement)

06. LUKASKI, H.C., HAYMES, E. and KANTER, M. 1998. Vitamin and mineral supplements and exercise. American College of Sports Medicine. May. Indianapolis, IN. (Policy statement)

Approved: FORREST H NIELSEN Date: 01/99
Title: CENTER DIRECTOR

INTERIM AND FINAL PROGRESS REPORTS

OF

TERMINATED CRIS WORK UNITS

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0146178 Year: 98 Project Number: 5450-51520-011-02 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.4 100%

Title: THE NUTRITIONAL ROLE OF BORON IN THE INHIBITION OF SERINE PROTEASES

Period Covered From: 01/98 To: 04/98

Progress Report
Experiments are being planned to isolate boron interactive biomolecules important in the control of the inflammatory process based on previous findings made by the staff assigned to this project that dietary boron fed in physiological concentrations alleviates induced arthritis and increases immunity. This project was inactive from January 1, to March 1, 1998 because of the departure of the research associate assigned to the project.

Publications:
Approved: FORREST H NIELSEN Date: 06/98
Title: CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0146178 Year: XX Project Number: 5450-51520-011-02 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.4 100%

Title: THE NUTRITIONAL ROLE OF BORON IN THE INHIBITION OF SERINE PROTEASES

Period Covered From: 04/93 To: 04/98

Progress Report

Experiments were conducted to test the hypothesis that dietary boron has a role in the prevention of inflammatory diseases, including arthritis, and in the modulation of immunity. In a rat model, rats were fed diets with either low or increased amounts of boron and then injected with a biological agent that induces arthritis. The results indicated that boron reduced the severity of induced arthritis and increased immunity against the injected inflammatory agent. Adding amounts of dietary boron typically found in bone tissue enhanced the multiplication of T lymphocytes but not B lymphocytes. Higher amounts of boron reduced the multiplication of both T and B lymphocytes. Other findings indicated that normal amounts of boron in the diet enhanced the production of antibodies by B lymphocytes. Boron fed to vitamin D-deficient chicks increased the concentrations of serum calcium and the active form of vitamin D in those animals. These findings suggest that normal amounts of boron in the diet improve immunity by affecting events early in the immune response process and enhances vitamin D availability. The above will be of value to scientists and nutrition specialists.

Publications:

Approved: FORREST H NIELSEN Date: 06/98
Title: CENTER DIRECTOR